

Transcription into the S/MAR element was found essential for stable maintenance of a corresponding vector (Rupprecht et al. (2010), Gene 466(1-2):36). S/MAR sequences as part of a transcription unit, i.e. S/MAR sequences downstream of a promoter, were proposed e.g. in WO 2019/057773 A1, WO 2019/057774 A1, and WO 2019/060253 A1. Epigenetic effects having an influence on replication of S/MAR vectors were identified (Haase et al.(2013), PLOS One 8(11):e79262).

Nonetheless, improved S/McAR-based vectors being stable enough for use stable modification of cells, e.g. for gene therapy, are still desirable.

SUMMARY

In one aspect, the present disclosure relates to a therapeutic cell comprising an episomal polynucleotide comprising a promoter and an expressible sequence, wherein said episomal polynucleotide further comprises an S/MAR element upstream of said promoter.

In another aspect, the present disclosure relates to an expression construct comprising an expressible sequence and a promoter, wherein said expression construct further comprises an S/MAR element upstream of said promoter, and wherein said expression construct replicates episomally in a mammalian cell.

In another aspect, the present disclosure relates to a polynucleotide comprising an S/MAR element, wherein said S/MAR element comprises a nucleic acid sequence being at least 93%, preferably at least 95%, identical to SEQ ID NO:3.

In another aspect, the present disclosure relates to an animal host cell comprising an S/MAR element, wherein said S/MAR element comprises a nucleic acid sequence being at least 70% identical to SEQ ID NO:1 and/or a nucleic acid sequence being at least 70% identical to SEQ ID NO:2.

In another aspect, the present disclosure relates to the therapeutic cell, the expression construct, the polynucleotide, or the animal host cell of the disclosure for use as a medicament.

In another aspect, the present disclosure relates to the use of the therapeutic cell, the expression construct, the polynucleotide, or the animal host cell of the disclosure in the manufacture of a medicament for immunotherapy and/or treating a genetic disease in a subject.

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DETAILED DESCRIPTION

In accordance, the present invention relates to a therapeutic cell comprising an episomal polynucleotide comprising a promoter and an expressible sequence, wherein said episomal polynucleotide further comprises an S/MAR element upstream of said promoter.

10 In general, terms used herein are to be given their ordinary and customary meaning to a person of ordinary skill in the art and, unless indicated otherwise, are not to be limited to a special or customized meaning. As used in the following, the terms “have”, “comprise” or “include” or any arbitrary grammatical variations thereof are used in a non-exclusive way. Thus, these terms
15 features are present in the entity described in this context and to a situation in which one or more further features are present. As an example, the expressions “A has B”, “A comprises B” and “A includes B” may both refer to a situation in which, besides B, no other element is present in A (i.e. a situation in which A solely and exclusively consists of B) and to a situation in which, besides B, one or more further elements are present in entity A, such as element C, elements C
20 and D or even further elements. Also, as is understood by the skilled person, the expressions "comprising a" and "comprising an" preferably refer to "comprising one or more", i.e. are equivalent to "comprising at least one".

Further, as used in the following, the terms "preferably", "more preferably", "most preferably",
25 "particularly", "more particularly", "specifically", "more specifically" or similar terms are used in conjunction with optional features, without restricting further possibilities. Thus, features introduced by these terms are optional features and are not intended to restrict the scope of the claims in any way. The invention may, as the skilled person will recognize, be performed by using alternative features. Similarly, features introduced by "in an

Claims

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1. A therapeutic cell comprising an episomal polynucleotide comprising a promoter and an expressible sequence, wherein said episomal polynucleotide further comprises an S/MAR element upstream of said promoter.
- 10 2. The therapeutic cell of claim 1, wherein said expressible sequence encodes a therapeutic gene product, preferably a therapeutic polypeptide.
3. The therapeutic cell of claim 1 or 2, wherein said expressible sequence encodes a therapeutic polypeptide, and wherein said therapeutic polypeptide is an antibody, a T
15 Cell Receptor (TCR), a Chimeric Antigen Receptor (CAR), a cytokine, and/or a polypeptide lacking in cells affected with a genetic disease.
4. The therapeutic cell of any one of claims 1 to 3, wherein said therapeutic polypeptide is a TCR.
- 20 5. The therapeutic cell of any one of claims 1 to 3, wherein said therapeutic polypeptide is a CAR.
6. The therapeutic cell of any one of claims 1 to 3, wherein said therapeutic polypeptide is
25 a polypeptide lacking in cells affected with a genetic disease.
7. The therapeutic cell of any one of claims 1 to 6, wherein said episomal polynucleotide further comprises a selectable marker gene.
- 30 8. The therapeutic cell of any one of claims 1 to 7, wherein said S/MAR element comprises a nucleic acid sequence being at least 70% identical to SEQ ID NO:1.
9. The therapeutic cell of any one of claims 1 to 8, wherein said S/MAR element comprises a nucleic acid sequence being at least 70% identical to SEQ ID NO:2.

10. The therapeutic cell of any one of claims 1 to 9, wherein said S/MAR element comprises a nucleic acid sequence being at least 70% identical to SEQ ID NO:3.
- 5 11. The therapeutic cell of any one of claims 1 to 10, wherein said therapeutic cell or host cell is a vertebrate cell, preferably a mammalian cell, more preferably a human cell.
12. An expression construct comprising an expressible sequence and a promoter, wherein said expression construct further comprises an S/MAR element upstream of said promoter, and wherein said expression construct replicates episomally in a mammalian
10 cell.
13. A polynucleotide comprising an S/MAR element, wherein said S/MAR element comprises a nucleic acid sequence being at least 93%, preferably at least 95%, identical to SEQ ID NO:3.
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14. The polynucleotide of claim 10, further comprising an expressible sequence and a promoter, preferably a mammalian promoter, wherein said S/MAR sequence is located upstream of said promoter.
- 20 15. An animal host cell comprising an S/MAR element, wherein said S/MAR element comprises a nucleic acid sequence being at least 70% identical to SEQ ID NO:1 and/or a nucleic acid sequence being at least 70% identical to SEQ ID NO:2.
- 25 16. The animal host cell of claim 15 being a therapeutic cell according to any one of claims 1 to 8.
17. The therapeutic cell of claims 1 to 11, the expression construct of claim 12, the polynucleotide of claims 13 to 14, or the animal host cell of claim 15 to 16 for use as a medicament.
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18. Use of the therapeutic cell of claims 1 to 11, the expression construct of claim 12, the polynucleotide of claims 13 to 14, or the animal host cell of claim 15 to 16 in the

manufacture of a medicament for immunotherapy and/or treating a genetic disease in a subject.

19. The use of claim 18 in immunotherapy, wherein said immunotherapy is treating and/or
5 preventing cancer and/or autoimmune disease by modulation of the immune response of a subject.
20. The use of claim 18 for treating genetic disease, wherein the genetic disease is a
10 monogenic recessive disease, preferably is phenylketonuria, alkaptonuria, Leber's Congenital Amaurosis, Choroideremia, or Stargardt disease.