TALENs. However, the latter are relatively ineffective at inserting the CAR gene into the T-cells.

[0006] Other systems are also used, such as viral vectors (AAV, Lentivirus) and the *sleeping beauty* transposon, but the pseudo-specific integration of the CAR gene with these approaches can lead to consequences downstream of their use (e.g. application WO2017180989A2).

[0007] Also, given the power of CAR technology, there is still a need for more efficient production of CAR-T modified T-cells.

[0008] The aim of the present invention is to overcome these drawbacks.

[0009] One aim of the invention is to propose a composition capable of enabling the simple and efficient production of CAR-T modified T-cells.

[0010] A further aim of the invention is to provide a method or a drug that is capable of treating diseases via said CAR-T modified T-cells.

SUMMARY

[0010a] In one aspect, provided herein is a composition comprising a first single-stranded nucleic acid molecule comprising or consisting essentially of an A sequence allowing the insertion of a complementary sequence of a nucleic acid of interest, or comprising a complementary sequence of a nucleic acid of interest, said complementary sequence binding at 5′ to a first T-rich sequence of 40 to 60 nucleotides in length and at 3′ to a second T-rich sequence of 40 to 60 nucleotides in length, said first and second T-rich sequences respectively comprising a first and a second domain of 6 to 12 G/C-rich nucleotides, the sequence of the first domain being complementary to the sequence of the second domain, said first and second domains being positioned 15 to 52 nucleotides from said A sequence, said first molecule

comprising at its 5′ end at least one first sequence oriented 5′-to-3′ for recognizing a transposase and at its 3′ end a second sequence for recognizing said transposase, a second single-stranded nucleic acid molecule comprising or consisting essentially of a B sequence allowing the insertion of a complementary sequence of a nucleic acid of interest, or comprising a complementary sequence of a nucleic acid of interest, said complementary B sequence binding at 5′ to a third T-rich sequence of 40 to 60 nucleotides in length and at 3′ to a fourth T-rich sequence of 40 to 60 nucleotides in length, said third and fourth T-rich sequences respectively comprising a third and a fourth domain of 6 to 12 G/C-rich nucleotides, the sequence of the third domain being complementary to the sequence of the fourth domain, said third and fourth domains being positioned 15 to 52 nucleotides from said B sequence, said second molecule comprising at its 5′ end at least one first sequence oriented 5′-to-3′ for recognizing said transposase and at its 3′ end the second sequence for recognizing said transposase, said B sequence being a complementary sequence of said nucleic acid of interest, the A sequence being positioned at 5′ of a region of interest of said nucleic acid of interest and the B sequence positioned at 3′ of the region of interest of said nucleic acid of interest, and a third single-stranded molecule comprising in its 5′ part, at least one complementary sequence of said second sequence for recognizing said transposase of the first molecule, in its 3′ part, at least one complementary sequence of said first sequence for recognizing said transposase of the second molecule, and an intermediate region located between the complementary sequence of said second sequence for recognizing said transposase of the first molecule and the complementary sequence of said first sequence for recognizing said transposase of the second molecule, said intermediate region comprising a sequence encoding a fusion protein, said fusion protein comprising, in the amino-terminal part, a peptide capable of binding to a membrane protein of a cell and, in the carboxy-terminal part, the activation domain of the T-lymphocyte, i.e. the CD3ζ chain of the T-cell receptor or TCR, the first and third single-stranded nucleic acid molecules being paired according to the base complementarity defined by Watson and

Crick so as to define two double-stranded binding sites of said transposase, and the second and third single-stranded nucleic acid molecules being paired according to the base complementarity defined by Watson and Crick so as to define two double-stranded binding sites of said transposase.

[0010b] In another aspect, provided herein is an isolated and purified immune cell, especially a T-lymphocyte cell, comprising a composition as described herein.

[0010c] In another aspect, provided herein is a pharmaceutical composition comprising: a composition as described herein, or an immune cell as described herein, in association with a pharmaceutically acceptable carrier.

[0010d] In another aspect, provided herein is a use of a composition as described herein, for inserting, into a T-lymphocyte cell, a sequence encoding a fusion protein comprising, in the amino-terminal part, a peptide capable of binding to a membrane protein of a cell and, in the carboxy-terminal part, the CD3ζ chain of the TCR, provided that the use does not comprise a process for modifying the germline genetic identity of human beings and that said use is not a method for treating the human or animal body by surgery or therapy.

[0010e] In another aspect, provided herein is a method for *in vitro* insertion of a sequence encoding a fusion protein comprising, in the amino-terminal part, a peptide capable of binding to a membrane protein of a cell and, in the carboxy-terminal part, the CD3ζ chain of the TCR, said method comprising: placing a composition as described herein in contact with a target nucleic acid comprising at least a 5′ region of the locus of the gene encoding the TRAC protein, in order to obtain a replacement complex, said composition being such that the A sequence of said first molecule comprises a complementary sequence of a first region of the locus of the gene encoding the TRAC protein, the B sequence of said second molecule comprises a complementary sequence of a second region of the locus of the gene encoding

the TRAC protein, and the third molecule comprises the sequence of a fusion protein comprising, in the amino-terminal part, a peptide capable of binding to a membrane protein of a cell and, in the carboxy-terminal part, the CD3ζ chain of the TCR located between a complementary sequence of said second sequence for recognizing said transposase of the first molecule and the complementary sequence of said first sequence for recognizing said transposase of the second molecule, placing the replacement complex in the presence of a transposase recognizing the double-stranded binding sites of said transposase contained in said composition, to obtain a recombination complex, and recombining the combination complex to obtain the sequence encoding the fusion protein inserted in the target nucleic acid between the first and second regions of the locus of the gene encoding the TRAC protein.

[0010f] In another aspect, provided herein is a method for producing chimeric antigenic receptor-T cells or *CAR-T cells* comprising the sequence of a fusion protein comprising, in the amino-terminal part, a peptide capable of binding to a membrane protein of a cell and, in the carboxy-terminal part, the CD3ζ chain of the TCR, said method comprising: placing a composition as described herein in contact with a purified T-cell, the genomic DNA of said purified T-cell comprising the locus of the gene encoding the TRAC protein, said composition or said ensemble being such that the A sequence of said first molecule comprises a complementary sequence of a first region of the locus of the gene encoding the TRAC protein, the B sequence of said second molecule comprises a complementary sequence of a second region of the locus of the gene encoding the TRAC protein, and the third molecule comprises the sequence of a fusion protein comprising, in the amino-terminal part, a peptide capable of binding to a membrane protein of a cell and, in the carboxy-terminal part, the CD3ζ chain of the TCR located between a complementary sequence of said second sequence for recognizing said transposase of the first molecule and the complementary sequence of said first sequence for recognizing said transposase of the second molecule, in order to obtain a replacement complex, placing the replacement complex in the

presence of a transposase recognizing the double-stranded binding sites of said transposase contained in said ensemble, to obtain a recombination complex, recombining the combination complex to obtain a *CAR-T* cell, the genomic DNA of said CAR-T cell comprising the sequence encoding said fusion protein at the locus of the gene encoding the TRAC protein, and purifying the *CAR-T* cell.

[0010g] In yet another aspect, provided herein is a modified T lymphoid cell capable of being obtained by the method as described herein, said modified T lymphoid cell comprising in its genome an insertion of a sequence encoding a fusion protein comprising, in the amino-terminal part, a peptide capable of binding to a membrane protein of a cell and, in the carboxy-terminal part, the CD3ζ chain of the TCR, said insertion being located at the locus of the gene encoding the TRAC protein.

[0011] The invention relates to a composition comprising

[0012] - a first single-stranded nucleic acid molecule comprising or consisting essentially of an A sequence allowing the insertion of a complementary sequence of a nucleic acid of interest, or comprising a complementary sequence of a nucleic acid of interest, said complementary sequence binding at 5′ to a first T-rich sequence of 40 to 60 nucleotides in length and at 3′ to a second T-rich sequence of 40 to 60 nucleotides in length, said first and second T-rich sequences respectively comprising a first and a second domain of 6 to 12 G/C-rich nucleotides, the sequence of the first domain being complementary to the sequence of the second domain, said first and second domains being positioned 15 to 52 nucleotides from said A sequence, said first molecule comprising at its 5′ end at least one first sequence oriented 5′-to-3′