

a DNA encoding a transposable element containing an expression cassette
for a transgene and
a nucleic acid encoding a transposase
into a mammalian cell,
thereby obtaining the recombinant mammalian cell,
wherein the DNA encoding the transposable element lacks an origin of replication and/or
lacks an antibiotic resistance gene,
wherein the nucleic acid encoding the transposase is an mRNA encoding the transposase,
and wherein the mammalian cell is a human lymphocyte.

In another aspect, the present disclosure refers to a composition comprising a minicircle
DNA encoding a transposable element and a nucleic acid encoding the transposase, wherein
the nucleic acid encoding the transposase is as defined herein; and wherein the
transposable element is as defined herein.

Claims

1. *An in vitro* use of
 - a combination of a minicircle DNA encoding a transposable element and
 - a source of a transposaseto stably integrate the transposable element into the genome of a mammalian cell,
wherein the source of the transposase is an mRNA encoding the transposase or wherein the source of the transposase is a transposase polypeptide, and
wherein the mammalian cell is a human lymphocyte.
2. The *in vitro* use according to claim 1, wherein the transposase is SB100X.
3. The *in vitro* use according to claim 1 or claim 2, wherein the transposable element contains the genetic information for the expression of a T-cell receptor or chimeric antigen receptor, and wherein the mammalian cell is a human T lymphocyte, preferably wherein the T-cell receptor or chimeric antigen receptor is tumor-reactive, and wherein the human T lymphocyte obtained by the use is a tumor-reactive human T lymphocyte suitable for use in the adoptive immunotherapy of cancer, and/or wherein the transposable element contains the genetic information for a chimeric antigen receptor, preferably, wherein the chimeric antigen receptor is specific for CD19, CD20, CD22, CD33, CD44v6, CD123, CD135, EpCAM, EGFR, an EGFR variant, GD2, ROR1, ROR2, CD269, CD319, CD38, or CD138.
4. The *in vitro* use according to any one of claims 1 to 3, wherein
 - I) the minicircle DNA encoding the transposable element encodes an a/b or g/d T-cell receptor, a cytokine, a suicide gene, or a transduction marker; and/or
 - II) the nucleic acid encoding the transposase and the DNA minicircle encoding the transposable element are introduced into the cell by electrotransfer, such as electroporation, nucleofection; chemotransfer, calcium phosphate; or nanoparticles; and/or
 - III) the transposase mediating transposition of the transposable element into the genome is Sleeping Beauty, PiggyBac, Frog Prince, Himarl, Passport, Minos, hAT, Tol1, Tol2, AciDs, PIF, Harbinger, Harbinger3-DR, and Hsmar1, or a derivative thereof having transposition activity.
5. *An in vitro* use of a combination of:
 - a DNA encoding a transposable element containing an expression cassette for a transgene and
 - a source of a transposaseto stably integrate the transposable element into the genome of a mammalian cell,

- wherein the DNA encoding the transposable element lacks an origin of replication and/or lacks an antibiotic resistance gene,
- wherein the source of the transposase is an mRNA encoding the transposase or wherein the source of the transposase is a transposase polypeptide,
- and wherein the mammalian cell is a human lymphocyte.
6. The *in vitro* use according to claim 5, wherein:
- I) the DNA encoding the transposable element is obtainable by deleting said origin of replication and/or said antibiotic resistance gene from a plasmid selected from the group consisting of:
 - pT;
 - pT2;
 - a plasmid having a DNA sequence which is at least 90% identical to the DNA sequence of pT;
 - a plasmid having a DNA sequence which is at least 90% identical to the DNA sequence of pT2; and
 - any other plasmid which is suitable as a donor plasmid for transposable elements; and/or
 - II) the total length of said DNA encoding the transposable element is not more than 3.0 kb, preferably not more than 2.0 kb greater than the length of said expression cassette; and/or
 - III) the total length of said DNA encoding the transposable element is not more than 1.5 kb greater than the length of said expression cassette; and/or
 - IV) the total length of said DNA encoding the transposable element is not more than 1.0 kb greater than the length of said expression cassette; and/or
 - V) the transgene is a T-cell receptor or chimeric antigen receptor as defined in claim 3, and wherein the mammalian cell is a human T lymphocyte; and/or
 - VI) the transgene is an a/b or g/d T-cell receptor, a cytokine, a suicide gene, or a transduction marker; and/or
 - VII) the use is a non-viral use.
7. An *in vitro* method for obtaining a recombinant mammalian cell containing a stably integrated transposable element, the method comprising:
- introducing a combination of a minicircle DNA encoding the transposable element and a nucleic acid encoding a transposase into a mammalian cell,

thereby obtaining the recombinant mammalian cell, wherein the nucleic acid encoding the transposase is an mRNA encoding the transposase; and/or the transposase is SB100X and is optionally delivered as a polypeptide,
and wherein the mammalian cell is a human lymphocyte.

8. The *in vitro* method according to claim 7, wherein the transposable element contains the genetic information for the expression of a T-cell receptor or chimeric antigen receptor, and wherein the mammalian cell is a human T lymphocyte, preferably wherein the T-cell receptor or chimeric antigen receptor is tumor-reactive, and wherein the human T lymphocyte obtained by the method is a tumor-reactive human T lymphocyte suitable for use in the adoptive immunotherapy of cancer.
9. The *in vitro* method according to claim 7 or claim 8, wherein the transposable element contains the genetic information for a chimeric antigen receptor, preferably wherein the chimeric antigen receptor is specific for CD19, CD20, CD22, CD33, CD44v6, CD123, CD135, EpCAM, EGFR, an EGFR variant, GD2, ROR1, ROR2, CD269, CD319, CD38, or CD138.
10. The *in vitro* method according to any one of claims 7 to 9, wherein:
 - I) the minicircle DNA encoding the transposable element encodes an a/b or g/d T-cell receptor, a cytokine, a suicide gene, or a transduction marker; and/or
 - II) the nucleic acid encoding the transposase and the DNA minicircle encoding the transposable element are introduced into the cell by electrotransfer, such as electroporation, nucleofection; chemotransfer, calcium phosphate; or nanoparticles; and/or
 - III) the transposase mediating transposition of the transposable element into the genome is Sleeping Beauty, PiggyBac, Frog Prince, Himarl, Passport, Minos, hAT, Tol1, Tol2, AciDs, PIF, Harbinger, Harbinger3-DR, and Hsmar1, or a derivative thereof having transposition activity; and/or
 - IV) the combination of the minicircle DNA encoding the transposable element and the nucleic acid encoding the transposase are introduced together into the mammalian cell; and/or
 - V) the nucleic acid encoding the transposase and the minicircle DNA encoding the transposable element are introduced into the mammalian cell in a molar ratio of 1:1 or more, preferably in a molar ratio of 2:1 to 10:1, more preferably in a molar ratio of 3:1 to 9:1, still more preferably in a molar ratio of 4:1 to 8:1.
11. An *in vitro* method for obtaining a recombinant mammalian cell containing a stably integrated transposable element, the method comprising:

introducing a combination of:

a DNA encoding a transposable element containing an expression cassette for a transgene and

a nucleic acid encoding a transposase

into a mammalian cell,

thereby obtaining the recombinant mammalian cell,

wherein the DNA encoding the transposable element lacks an origin of replication and/or lacks an antibiotic resistance gene,

wherein the nucleic acid encoding the transposase is an mRNA encoding the transposase,

and wherein the mammalian cell is a human lymphocyte.

12. The *in vitro* method according to claim 11, wherein the DNA encoding the transposable element is obtainable by deleting said origin of replication and/or said antibiotic resistance gene from a plasmid selected from the group consisting of:

pT;

pT2;

a plasmid having a DNA sequence which is at least 90% identical to the DNA sequence of pT;

a plasmid having a DNA sequence which is at least 90% identical to the DNA sequence of pT2;
and

any other plasmid which is suitable as a donor plasmid for transposable elements.

13. The *in vitro* method according to claim 12, wherein:

I) the total length of said DNA encoding the transposable element is not more than 3.0 kb, preferably not more than 2.0 kb greater than the length of said expression cassette; and/or

II) the total length of said DNA encoding the transposable element is not more than 1.5 kb greater than the length of said expression cassette; and/or

III) the total length of said DNA encoding the transposable element is not more than 1.0 kb greater than the length of said expression cassette; and/or

IV) the DNA encoding the transposable element is a minicircle DNA as used in any one of the preceding claims; and/or

V) the transgene is a T-cell receptor or chimeric antigen receptor as defined in any one of claims 8 or 9, and wherein the mammalian cell is a human T lymphocyte or the transgene is an a/b or g/d T-cell receptor, a cytokine, a suicide gene, or a transduction marker; and/or

VI) the method is a non-viral method.

14. The *in vitro* method according to any one of claims 7 to 13, wherein said combination is introduced by introducing
- said nucleic acid encoding the transposase
 - and said DNA or minicircle DNA encoding the transposable element
- simultaneously; or
- said combination is introduced by introducing
- said DNA or minicircle DNA encoding the transposable element; and
 - said nucleic acid encoding the transposase sequentially.
15. The *in vitro* method according to any one of claims 7 to 14 or the *in vitro* use according to any one of claims 1 to 6, wherein:
- I) the minicircle DNA encoding the transposable element is a linearized DNA or a circular DNA; and/or
 - II) the nucleic acid encoding the transposase and the DNA or minicircle DNA encoding the transposable element are introduced into the mammalian cell in a weight ratio of 1:1 or more, preferably in a weight ratio of 2:1 to 10:1, more preferably in a weight ratio of 3:1 to 9:1, still more preferably in a weight ratio of 4:1 to 8:1.
16. A composition comprising a minicircle DNA encoding a transposable element and a nucleic acid encoding the transposase, wherein the nucleic acid encoding the transposase is as defined in claim 1; and wherein the transposable element is as defined in claims 3 or 4.
17. The *in vitro* use of any one of claims 1 to 6, or the *in vitro* method of any one of claims 7 to 15, wherein the cell is a human T lymphocyte; or
- wherein the cell is a CD8⁺ killer T cell, a CD4⁺ helper T cell, a naive T cell, a memory T cell, a central memory T cell, an effector memory T cell, a memory stem T cell, an invariant T cell, an NKT cell, a cytokine induced killer T cell, a g/d T cell, a B lymphocyte, or a natural killer cell.