

METHODS AND COMPOSITIONS FOR GENOMIC INTEGRATION

CROSS REFERENCE

[0001] This application claims the benefit of priority to U.S. Provisional Application No. 63/187,117, filed on May 11, 2021, U.S. Provisional Application No. 63/254,791, filed on October 12, 2021, and U.S. Provisional Application No. 63/274,907, filed on November 2, 2021, each of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Cell therapy is a rapidly developing field for addressing difficult to treat diseases, such as cancer, persistent infections and certain diseases that are refractory to other forms of treatment. Cell therapy often utilizes cells that are engineered ex vivo and administered to an organism to correct deficiencies within the body. An effective and reliable system for manipulation of a cell's genome is crucial, in the sense that when the engineered cell is administered into an organism, it functions optimally and with prolonged efficacy. Likewise, reliable mechanisms of genetic manipulation form the cornerstone in the success of gene therapy. However, severe deficiencies exist in methods for delivering nucleic acid cargo (e.g., large cargo) in a therapeutically safe and effective manner. Viral delivery mechanisms are frequently used to deliver large nucleic acid cargo in a cell but are tied to safety issues and cannot be used to express the cargo in some cell types. Additionally, subjecting a cell to repeated gene manipulation can affect cell health, induce alterations of cell cycle and render the cell unsuitable for therapeutic use. Advancements are continually sought in the area for efficacious delivery and stabilization of an exogenously introduced genetic material for therapeutic purposes.

SUMMARY

[0003] Provided herein is a pharmaceutical composition comprising a therapeutically effective amount of one or more polynucleic acids, or at least one vector encoding the one or more polynucleic acids, the one or more polynucleic acids comprising: a mobile genetic element comprising a sequence encoding a polypeptide; and an insert sequence, wherein the insert sequence comprises a sequence that is a reverse complement of a sequence encoding an exogenous therapeutic polypeptide, wherein the polypeptide encoded by the sequence of the mobile genetic element promotes integration of the insert sequence into a genome of a cell; and wherein the pharmaceutical composition is substantially non-immunogenic to a human subject.

[0003a] In one aspect, provided herein is use of a population of human cells in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an autoimmune disease and an infectious disease, wherein the population of human cells is to be contacted with one or more polynucleic acids comprising:

- (a) a sequence encoding long interspersed nuclear element (LINE)-1 polypeptides comprising:
 - (i) a human ORF1p, and

- (ii) a human ORF2p, wherein the human ORF2p comprises a first nuclear localization signal (NLS) from SV40 and a second NLS from nucleoplasmin, and wherein the human ORF2p comprises an endonuclease domain and a reverse transcriptase domain;
- (b) a sequence encoding a Cas9 endonuclease, wherein the Cas9 endonuclease is a Cas9 nickase,
- (c) a guide RNA sequence, or a polynucleic acid encoding the guide RNA sequence; wherein the guide RNA sequence targets a DNA sequence in a genomic target site of the genome of a cell of the population of the human cells;
- (d) an insert sequence, wherein the insert sequence is a reverse complement of a sequence comprising a gene encoding an exogenous therapeutic polypeptide, wherein the polypeptides encoded by the sequence of the LINE-1 polypeptides promotes integration of the sequence comprising the gene into the genomic target site of the genome of the cell of the population of human cells via target-primed reverse transcription (TPRT),

and wherein the one or more polynucleic acids comprise one or more mRNA molecules, and wherein the population of human cells is modified.

[0003b] In another aspect, provided herein is a composition comprising one or more polynucleic acids comprising one or more mRNA molecules, comprising:

- (a) a 5'UTR sequence;
- (b) a mobile genetic element, wherein the mobile genetic element comprises a sequence encoding LINE-1 polypeptides comprising:
 - (i). a human ORF1p, and
 - (ii). a human ORF2p, wherein the human ORF2p comprises a first NLS from SV40 and a second NLS from nucleoplasmin, and wherein the human ORF2p comprises an endonuclease domain and a reverse transcriptase domain;
- (c) a guide RNA sequence, or a polynucleic acid encoding the guide RNA sequence; wherein the guide RNA sequence targets a DNA sequence in a genomic target site of the genome of a cell of the population of the human cells;
- (d) a sequence encoding a Cas9 endonuclease, wherein the Cas9 endonuclease is a Cas9 nickase,
- (e) an insert sequence, wherein the insert sequence is a reverse complement of a sequence comprising a gene encoding an exogenous therapeutic polypeptide; and
- (f) a 3'UTR sequence downstream of the insert sequence.

[0003c] In another aspect, provided herein is use of an exogenous human therapeutic polypeptide in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an autoimmune disease and an infectious disease, wherein the exogenous human

therapeutic polypeptide is encoded by an exogenous sequence from a site-specifically integrated genomic DNA sequence of a target human cell, wherein

(a) the target human cell is to be contacted with a composition, the composition comprising one or more RNA molecules comprising (i) a first RNA molecule encoding a Cas nickase (ii) a second RNA molecule, and (iii) a guide RNA or a nucleic acid encoding the guide RNA, wherein the target human cell uptakes the one or more RNA molecules, and the second RNA molecule comprises:

(i) a sequence that is a reverse complement of a DNA sequence that encodes the exogenous human therapeutic polypeptide; and

(ii).a mobile genetic element, wherein the mobile genetic element comprises an RNA sequence encoding a polypeptide with target-primed reverse transcription (TPRT) activity, wherein the polypeptide with TPRT activity comprises an endonuclease with a mutation that abrogates endonuclease activity of the endonuclease domain, and wherein the polypeptide with TPRT activity comprises a first NLS from SV40 and a second NLS from nucleoplasmin;

(b) the RNA sequence encoding the polypeptide with TPRT activity and the RNA sequence encoding the Cas nickase is to be translated;

(c) the genomic DNA site of the target human cell via the Cas nickase translated in (b) is to be nicked;

(d) the sequence that is the reverse complement of the DNA sequence that encodes the human therapeutic polypeptide via the TPRT activity of the polypeptide with TPRT activity translated in (b) is to be reversed transcribed for production of a DNA sequence encoding the exogenous human therapeutic polypeptide;

(e) the DNA sequence encoding the exogenous human therapeutic polypeptide produced in (d) is to be integrated into a genomic DNA site of the target human cell; and

(f) the exogenous human therapeutic polypeptide is to be expressed in the target human cell, wherein the exogenous human therapeutic polypeptide is expressed from the DNA sequence integrated into the genomic DNA of the target human cell in (e).

[0003d] In another aspect, provided herein is a composition comprising:

(i) an RNA molecule encoding a Cas nickase;

(ii) a guide RNA or a nucleic acid encoding the guide RNA and

(iii) one or more RNA molecules, comprising

(a) a sequence that is a reverse complement of a DNA sequence that encodes an exogenous human therapeutic polypeptide; and

(b) a mobile genetic element, wherein the mobile genetic element comprises an RNA sequence encoding a polypeptide with target-primed reverse transcription (TPRT) activity,

wherein the polypeptide with TPRT activity comprises an endonuclease with a mutation that abrogates endonuclease activity of the endonuclease domain, and wherein the polypeptide with TPRT activity comprises a first NLS from SV40 and a second NLS from nucleoplasmin.

[0004] In some embodiments, the polypeptide encoded by the sequence of the mobile genetic element comprises one or more long interspersed nuclear element (LINE) polypeptides, wherein the one or more LINE polypeptides comprises: human ORF1p or a functional fragment thereof, and human ORF2p or a functional fragment thereof.

[0005] In some embodiments, the insert sequence stably integrates and/or is retrotransposed into the genome of a human cell.

CLAIMS

What is claimed is:

1. Use of a population of human cells in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an autoimmune disease and an infectious disease, wherein the population of human cells is to be contacted with one or more polynucleic acids comprising:
 - (a) a sequence encoding long interspersed nuclear element (LINE)-1 polypeptides comprising:
 - (i) a human ORF1p, and
 - (ii) a human ORF2p, wherein the human ORF2p comprises a first nuclear localization signal (NLS) from SV40 and a second NLS from nucleoplasmin, and wherein the human ORF2p comprises an endonuclease domain and a reverse transcriptase domain;
 - (b) a sequence encoding a Cas9 endonuclease, wherein the Cas9 endonuclease is a Cas9 nickase,
 - (c) a guide RNA sequence, or a polynucleic acid encoding the guide RNA sequence; wherein the guide RNA sequence targets a DNA sequence in a genomic target site of the genome of a cell of the population of the human cells;
 - (d) an insert sequence, wherein the insert sequence is a reverse complement of a sequence comprising a gene encoding an exogenous therapeutic polypeptide, wherein the polypeptides encoded by the sequence of the LINE-1 polypeptides promotes integration of the sequence comprising the gene into the genomic target site of the genome of the cell of the population of human cells via target-primed reverse transcription (TPRT), and wherein the one or more polynucleic acids comprise one or more mRNA molecules, and wherein the population of human cells is modified.
2. The use of claim 1, wherein the exogenous therapeutic polypeptide is selected from the group consisting of a ligand, an antibody, a receptor, an enzyme, a transport protein, a structural protein, a hormone, a contractile protein, a storage protein and a transcription factor.

3. The use of any one of claims 1-2, wherein the first NLS is an N-terminal NLS.
4. The use of any one of claims 1-3, wherein the second NLS is a C-terminal NLS.
5. The use of any one of claims 1-4, wherein the first NLS comprises an amino acid sequence of SEQ ID NO: 80 and the second NLS comprises an amino acid sequence of SEQ ID NO: 84.
6. The use of any one of claims 1-5, wherein the one or more polynucleic acids comprise a first and a second guide RNA sequence or a nucleic acid sequence encoding the first and the second guide RNA sequence, optionally wherein the first guide RNA sequence targets a DNA sequence upstream of the genomic target site and the second guide RNA sequence targets a DNA sequence downstream of the genomic target site.
7. The use of any one of claims 1-6, wherein the human ORF2p comprises a mutation that inactivates the activity of the ORF2p endonuclease domain, or reduces the DNA target recognition of ORF2p endonuclease compared to the nuclease domain without the mutation.
8. The use of claim 7, wherein the mutation comprises a D205 mutation of the endonuclease domain.
9. The use of any one of claims 1-8, wherein the sequence comprising the gene is integrated into the genome by TPRT following cleavage of a DNA strand of a target site by the Cas9 nickase.
10. The use of claim 1, wherein the one or more polynucleic acids comprises
 - (i) a recombinant RNA molecule comprising:
 - (a) a 5'UTR sequence comprising a sequence that is between 2-15 nucleotides in length and binds to a target sequence by homology or binds to a genomic DNA priming sequence;

(b) the insert sequence, wherein the insert sequence is downstream of the sequence encoding the LINE-1 polypeptides; and

(c) a 3'UTR sequence downstream of the insert sequence, and wherein the 3'UTR sequence comprises a sequence that binds to a target sequence by homology or a genomic DNA priming sequence.

11. The use of any one of claims 1-10, wherein the cancer is selected from the group consisting of liver cancer, breast cancer, ovarian cancer, skin cancer, lung cancer, pancreatic cancer, colorectal cancer, bladder cancer, and stomach cancer.

12. A composition comprising one or more polynucleic acids comprising one or more mRNA molecules, comprising:

(a) a 5'UTR sequence;

(b) a mobile genetic element, wherein the mobile genetic element comprises a sequence encoding LINE-1 polypeptides comprising:

(i) a human ORF1p, and

(ii) a human ORF2p, wherein the human ORF2p comprises a first NLS from SV40 and a second NLS from nucleoplasmin, and wherein the human ORF2p comprises an endonuclease domain and a reverse transcriptase domain;

(c) a guide RNA sequence, or a polynucleic acid encoding the guide RNA sequence; wherein the guide RNA sequence targets a DNA sequence in a genomic target site of the genome of a cell of the population of the human cells;

(d) a sequence encoding a Cas9 endonuclease, wherein the Cas9 endonuclease is a Cas9 nickase,

(e) an insert sequence, wherein the insert sequence is a reverse complement of a sequence comprising a gene encoding an exogenous therapeutic polypeptide; and

(f) a 3'UTR sequence downstream of the insert sequence.

13. The composition of claim 12, wherein

(a) the 5'UTR sequence comprises a sequence that is between 2-15 nucleotides in length and binds to a target sequence by homology or binds to a genomic DNA priming sequence; and

(b) the 3'UTR sequence comprises a sequence that binds to a target sequence by homology or a genomic DNA priming sequence.

14. The composition of claim 12 or 13, wherein the composition comprises a first and a second guide RNA sequence or a nucleic acid sequence encoding the first and the second guide RNA sequence, optionally wherein the first guide RNA sequence targets a DNA sequence upstream of the genomic target site and the second guide RNA sequence targets a DNA sequence downstream of the genomic target site.
15. The composition of any one of claims 12-14, wherein the human ORF2p comprises a mutation that inactivates the activity of the ORF2p endonuclease domain, or reduces the DNA target recognition of ORF2p endonuclease compared to the nuclease domain without the mutation.
16. The composition of claim 15, wherein the mutation comprises a D205 mutation.
17. The composition of any one of claims 12-16, wherein the first NLS is an N-terminal NLS.
18. The composition of any one of claims 12-17, wherein the second NLS is a C-terminal NLS.
19. The composition of any one of claims 12-18, wherein the first NLS comprises a sequence of SEQ ID NO: 80 and the second NLS comprises a sequence of SEQ ID NO: 84.
20. The composition of any one of claims 12-19, further comprising one or more siRNAs capable of knocking down any one of ADAR1, ADAR2 (ADAR1B), APOBEC3C, BRCA1, let-7 miRNA, RNase L, TASHOR (HUSH complex) and/or RAD51.
21. The composition of any one of claims 12-20, wherein the composition further comprises lipid nanoparticles (LNPs) encapsulating the one or more mRNA molecules.
22. Use of an exogenous human therapeutic polypeptide in the manufacture of a medicament for treating a disease selected from the group consisting of cancer, an autoimmune disease and an infectious disease, wherein the exogenous human therapeutic polypeptide

is encoded by an exogenous sequence from a site-specifically integrated genomic DNA sequence of a target human cell, wherein

- (a) the target human cell is to be contacted with a composition, the composition comprising one or more RNA molecules comprising (i) a first RNA molecule encoding a Cas nickase (ii) a second RNA molecule, and (iii) a guide RNA or a nucleic acid encoding the guide RNA, wherein the target human cell uptakes the one or more RNA molecules, and the second RNA molecule comprises:
 - (i) a sequence that is a reverse complement of a DNA sequence that encodes the exogenous human therapeutic polypeptide; and
 - (ii) a mobile genetic element, wherein the mobile genetic element comprises an RNA sequence encoding a polypeptide with target-primed reverse transcription (TPRT) activity, wherein the polypeptide with TPRT activity comprises an endonuclease with a mutation that abrogates endonuclease activity of the endonuclease domain, and wherein the polypeptide with TPRT activity comprises a first NLS from SV40 and a second NLS from nucleoplasmin;
- (b) the RNA sequence encoding the polypeptide with TPRT activity and the RNA sequence encoding the Cas nickase is to be translated;
- (c) the genomic DNA site of the target human cell via the Cas nickase translated in (b) is to be nicked;
- (d) the sequence that is the reverse complement of the DNA sequence that encodes the human therapeutic polypeptide via the TPRT activity of the polypeptide with TPRT activity translated in (b) is to be reversed transcribed for production of a DNA sequence encoding the exogenous human therapeutic polypeptide;
- (e) the DNA sequence encoding the exogenous human therapeutic polypeptide produced in (d) is to be integrated into a genomic DNA site of the target human cell; and
- (f) the exogenous human therapeutic polypeptide is to be expressed in the target human cell, wherein the exogenous human therapeutic polypeptide is expressed from the DNA sequence integrated into the genomic DNA of the target human cell in (e).

23. The use of claim 22, wherein the exogenous human therapeutic polypeptide is to be expressed in at least 2% of target human cells that are contacted with the composition.
24. The use of claim 22 or 23, wherein the second RNA molecule comprises a homology arm complementary to a sequence comprising the genomic DNA site of the target human cell.
25. The use of any one of claims 22-24, wherein the cancer is selected from the group consisting of liver cancer, breast cancer, ovarian cancer, skin cancer, lung cancer, pancreatic cancer, colorectal cancer, bladder cancer, and stomach cancer.
26. A composition comprising:
 - (i) an RNA molecule encoding a Cas nickase;
 - (ii) a guide RNA or a nucleic acid encoding the guide RNA and
 - (iii) one or more RNA molecules, comprising
 - (a) a sequence that is a reverse complement of a DNA sequence that encodes an exogenous human therapeutic polypeptide; and
 - (b) a mobile genetic element, wherein the mobile genetic element comprises an RNA sequence encoding a polypeptide with target-primed reverse transcription (TPRT) activity, wherein the polypeptide with TPRT activity comprises an endonuclease with a mutation that abrogates endonuclease activity of the endonuclease domain, and wherein the polypeptide with TPRT activity comprises a first NLS from SV40 and a second NLS from nucleoplasmin.