

examined the safety and efficacy of lentivirus-mediated gene therapy for RAG deficiency in preclinical models showing poor immune reconstitution or severe signs of inflammation, with cellular infiltrates in the skin, lung, liver, kidney, and presence of circulating anti-double strand DNA (van Til NP, et al. J Allergy Clin Immunol. 2014;133(4):1116–23).

- 5 Overall, these data raise significant concerns on the clinical use of conventional RAG1 gene therapy vectors that allow suboptimal levels and deregulated pattern of gene expression.

Thus, there is a demand for improved treatments for RAG1 deficiency.

## **SUMMARY OF THE INVENTION**

10 The present inventors have developed a gene editing strategy to correct mutations in the *RAG1* gene by targeting the genomic region located at the 5' of the second exon, which contains the entire coding sequence of the gene.

The present inventors have designed and selected a panel of CRISPR-Cas9 nucleases and identified specific sites in non-repeated regions of the first intron of the human *RAG1* gene. The present inventors have identified guide RNAs and optimal conditions for the delivery of the CRISPR-Cas9 nuclease ribonucleoprotein complexes. In parallel, the present inventors have developed a donor DNA carrying the human *RAG1* cDNA.

20 The gene editing strategy allows a high level of activity (measured as frequency of NHEJ-mutagenesis) and targeting efficiency (measured as GFP expression), both in a surrogate cell line deficient in RAG1 expression and expressing a recombination cassette, and in humans CD34+ HSCs obtained from mobilized peripheral blood (mPB). High editing efficiencies were reached in mobilized peripheral blood (mPB) CD34+ cells using the gene editing strategy.

In one aspect, the present ~~invention~~disclosure provides a polynucleotide comprising from 5' to 3': a first homology region, a splice acceptor sequence, a nucleotide sequence encoding a RAG1 polypeptide, and a second homology region.

25 In another aspect, the present ~~invention~~disclosure provides a polynucleotide comprising from 5' to 3': a first homology region, a nucleotide sequence encoding a RAG1 polypeptide, and a second homology region.

In some ~~embodiments~~examples:

30 (i) the first homology region is homologous to a first region of the RAG1 intron 1 and the second homology region is homologous to a second region of the RAG1 intron 1; or

(ii) the first homology region is homologous to a first region of the RAG1 intron 1 or the RAG1 exon 2 and the second homology region is homologous to a second region of the RAG1 exon 2.

5 In some ~~embodiments~~examples, the first homology region is homologous to a first region of the RAG1 intron 1 and the second homology region is homologous to a second region of the RAG1 intron 1.

In some ~~embodiments~~examples, the first homology region is homologous to a first region of the RAG1 intron 1 and the second homology region is homologous to a second region of the RAG1 exon 2.

10 In some ~~embodiments~~examples, the first homology region is homologous to a first region of the RAG1 exon 2 and the second homology region is homologous to a second region of the RAG1 exon 2.

In some ~~embodiments~~examples:

15 (i) the first homology region is homologous to a region upstream of chr 11: 36569295 and the second homology region is homologous to a region downstream of chr 11: 36569298;

(ii) the first homology region is homologous to a region upstream of chr 11: 36573790 and the second homology region is homologous to a region downstream of chr 11: 36573793;

20 (iii) the first homology region is homologous to a region upstream of chr 11: 36573641 and the second homology region is homologous to a region downstream of chr 11: 36573644;

25 (iv) the first homology region is homologous to a region upstream of chr 11: 36573351 and the second homology region is homologous to a region downstream of chr 11: 36573354;

(v) the first homology region is homologous to a region upstream of chr 11: 36569080 and the second homology region is homologous to a region downstream of chr 11: 36569083;

30 (vi) the first homology region is homologous to a region upstream of chr 11: 36572472 and the second homology region is homologous to a region downstream of chr 11: 36572475;

(vii) the first homology region is homologous to a region upstream of chr 11: 36571458 and the second homology region is homologous to a region downstream of chr 11: 36571461;

5 (viii) the first homology region is homologous to a region upstream of chr 11: 36571366 and the second homology region is homologous to a region downstream of chr 11: 36571369;

(ix) the first homology region is homologous to a region upstream of chr 11: 36572859 and the second homology region is homologous to a region downstream of chr 11: 36572862;

10 (x) the first homology region is homologous to a region upstream of chr 11: 36571457 and the second homology region is homologous to a region downstream of chr 11: 36571460;

(xi) the first homology region is homologous to a region upstream of chr 11: 36569351 and the second homology region is homologous to a region downstream of chr 11: 36569354; or  
15

(xii) the first homology region is homologous to a region upstream of chr 11: 36572375 and the second homology region is homologous to a region downstream of chr 11: 36572378.

| In some ~~embodiments~~examples:

20 (i) the first homology region is homologous to a region upstream of chr 11: 36569295 and the second homology region is homologous to a region downstream of chr 11: 36569298;

(ii) the first homology region is homologous to a region upstream of chr 11: 36573351 and the second homology region is homologous to a region downstream of chr 11: 36573354; or  
25

(iii) the first homology region is homologous to a region upstream of chr 11: 36571366 and the second homology region is homologous to a region downstream of chr 11: 36571369.

| In preferred ~~embodiments~~examples, the first homology region is homologous to a region  
30 upstream of chr 11: 36569295 and the second homology region is homologous to a region downstream of chr 11: 36569298.

In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36573790 and the second homology region is homologous to a region downstream of chr 11: 36573793.

5 In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36573641 and the second homology region is homologous to a region downstream of chr 11: 36573644.

In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36573351 and the second homology region is homologous to a region downstream of chr 11: 36573354.

10 In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36569080 and the second homology region is homologous to a region downstream of chr 11: 36569083.

15 In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36572472 and the second homology region is homologous to a region downstream of chr 11: 36572475.

In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36571458 and the second homology region is homologous to a region downstream of chr 11: 36571461.

20 In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36571366 and the second homology region is homologous to a region downstream of chr 11: 36571369.

In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36572859 and the second homology region is homologous to a region downstream of chr 11: 36572862.

25 In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36571457 and the second homology region is homologous to a region downstream of chr 11: 36571460.

30 In some ~~embedments~~examples, the first homology region is homologous to a region upstream of chr 11: 36569351 and the second homology region is homologous to a region downstream of chr 11: 36569354.

- In some ~~embodiments~~examples, the first homology region is homologous to a region upstream of chr 11: 36572375 and the second homology region is homologous to a region downstream of chr 11: 36572378.
- 5 In preferred ~~embodiments~~examples, the first homology region is homologous to a region comprising chr 11: 36569245-chr 11: 36569294 and/or the second homology region is homologous to a region comprising chr 11: 36569299-chr 11: 36569348.
- 10 In some ~~embodiments~~examples, the 3' terminal sequence of the first homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 7 and/or the 5' terminal sequence of the second homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 19.
- In some ~~embodiments~~examples, the first homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 31, or a fragment thereof and/or the second homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 32, or a fragment thereof.
- 15 In some ~~embodiments~~examples, the first and second homology regions are each 50-1000bp in length, 100-500 bp in length, or 200-400 bp in length.
- In some ~~embodiments~~examples, the nucleotide sequence encoding a RAG1 polypeptide comprises or consists of a nucleotide sequence encoding an amino acid sequence that has at least 70% identity to SEQ ID NO: 4 or SEQ ID NO: 5.
- 20 In some ~~embodiments~~examples, the nucleotide sequence encoding a RAG1 polypeptide comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 6.
- In some ~~embodiments~~examples, the splice acceptor site comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 33.
- 25 In preferred ~~embodiments~~examples, the nucleotide sequence encoding a RAG1 polypeptide is operably linked to a polyadenylation sequence, optionally wherein the polyadenylation sequence is a bGH polyadenylation sequence.
- 30 In some ~~embodiments~~examples, the nucleotide sequence encoding a RAG1 polypeptide is operably linked to a polyadenylation sequence comprising or consisting of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 35.

In some ~~embodiment~~examples, the nucleotide sequence encoding a RAG1 polypeptide is operably linked a Kozak sequence, optionally wherein the Kozak sequence comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 36.

5 In some ~~embodiment~~examples, the polynucleotide comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 39.

In another aspect, the present ~~invention~~disclosure provides a vector comprising the polynucleotide of the ~~invention~~disclosure.

10 In some ~~embodiment~~examples, the vector is a viral vector, optionally an adeno-associated viral (AAV) vector such as an AAV6 vector. In some ~~embodiment~~examples, the vector is a lentiviral vector, such as an integration-defective lentiviral vector (IDLV).

In another aspect, the present ~~invention~~disclosure provides a guide RNA comprising or consisting of a nucleotide sequence that has at least 90% identity to any of SEQ ID NOs: 41-52.

In another aspect, the present ~~invention~~disclosure provides a guide RNA comprising or consisting of a nucleotide sequence that has at least 90% identity to any of SEQ ID NOs: 53-55.

15 In preferred ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 41. In preferred ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 53. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 42. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 43. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 44. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 45. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 46. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 47. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 48. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 49. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 50. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 51. In some ~~embodiment~~examples, the guide RNA comprises or consists of a nucleotide sequence

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that has at least 90% identity to SEQ ID NO: 52. In some ~~embodiments~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 54. In some ~~embodiments~~examples, the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 55.

5 In some ~~embodiments~~examples, from one to five of the terminal nucleotides at 5' end and/or 3' end of the guide RNA are chemically modified to enhance stability, optionally wherein three terminal nucleotides at 5' end and/or 3' end if the guide RNA are chemically modified to enhance stability, optionally wherein the chemical modification is modification with 2'-O-methyl 3'phosphorothioate.

10 In another aspect, the present ~~invention~~disclosure provides a kit comprising the polynucleotide or the vector of the ~~invention~~disclosure.

In another aspect, the present ~~invention~~disclosure provides a composition comprising the polynucleotide or the vector of the ~~invention~~disclosure.

15 In another aspect, the present ~~invention~~disclosure provides a gene-editing system comprising the polynucleotide or the vector of the ~~invention~~disclosure.

In some ~~embodiments~~examples, the kit, composition, or gene-editing system further comprises a guide RNA of the ~~invention~~disclosure. In some ~~embodiments~~examples, the kit, composition, or gene-editing system further comprises a RNA-guided nuclease, optionally wherein the RNA-guided nuclease is a Cas9 endonuclease

20 In another aspect, the present ~~invention~~disclosure provides for use of the polynucleotide, the vector, the kit, the composition, or the gene-editing system, for gene editing a cell or a population of cells. In some ~~embodiments~~examples, the use is ex vivo or in vitro use.

In another aspect, the present ~~invention~~disclosure provides a genome comprising the polynucleotide of the ~~invention~~disclosure.

25 In another aspect, the present ~~invention~~disclosure provides a genome comprising a splice acceptor sequence and a nucleotide sequence encoding a RAG1 polypeptide located in the RAG1 intron 1 or RAG1 exon 2. In some ~~embodiments~~examples, the splice acceptor sequence and the nucleotide sequence encoding RAG1 are located in the RAG1 intron 1.

In some ~~embodiments~~examples:

30 (i) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36569295 to chr 11: 36569298;

- (ii) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36573790 to chr 11: 36573793;
- (iii) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36573641 to chr 11: 36573644;
- 5 (iv) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36573351 to chr 11: 36573354;
- (v) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36569080 to chr 11: 36569083;
- 10 (vi) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36572472 to chr 11: 36572475;
- (vii) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36571458 to chr 11: 36571461;
- (viii) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36571366 to chr 11: 36571369;
- 15 (ix) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36572859 to chr 11: 36572862;
- (x) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36571457 to chr 11: 36571460;
- 20 (xi) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36569351 to chr 11: 36569354; or
- (xii) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36572375 to chr 11: 36572378.

In some ~~embodiments~~examples:

- 25 (i) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36569295 to chr 11: 36569298;
- (ii) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36573351 to chr 11: 36573354; or

(iii) the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36571366 to chr 11: 36571369.

In some ~~embodiments~~examples, the splice acceptor sequence and the nucleotide sequence encoding RAG1 replace chr 11: 36569295 to chr 11: 36569298.

5 In another aspect, the present ~~invention~~disclosure provides a cell comprising the polynucleotide, the vector, or the genome of the ~~invention~~disclosure.

In another aspect, the present ~~invention~~disclosure provides a population of cells comprising one or more cells of the present ~~invention~~disclosure.

10 In another aspect, the present ~~invention~~disclosure provides a method of gene editing a population of cells comprising delivering the polynucleotide or the vector of the ~~invention~~disclosure to a population of cells to obtain a population of gene-edited cells. In some ~~embodiments~~examples, the method is an ex vivo or in vitro method.

In another aspect, the present ~~invention~~disclosure provides a method of treating immunodeficiency in a subject in need thereof, comprising delivering the polynucleotide or the vector of the ~~invention~~disclosure to a population of cells to obtain a population of gene-edited cells and administering the population of gene-edited cells to the subject.

15 In another aspect, the present disclosure provides a use of a population of gene-edited cells in the manufacture of a medicament for treating a RAG-deficient immunodeficiency in a subject, wherein the use comprises an RNA-guided nuclease, a guide RNA according to claim 19 or claim 20, and  
20 a vector according to claim 17 or claim 18, to be delivered to a population of cells to obtain the population of gene-edited cells, and wherein the population of gene-edited cells is to be administered to the subject.

In another aspect, the present ~~invention~~disclosure provides a population of gene-edited cells obtainable by the method of the ~~invention~~disclosure.

25 In another aspect, the present ~~invention~~disclosure provides the polynucleotide, the vector, the guide RNA, the kit, the composition, or the gene-editing system, for use in treating immunodeficiency in a subject.

In another aspect, the present ~~invention~~disclosure provides a method of treating a subject comprising administering a cell, a population of cells, or a population of gene edited cells of the present ~~invention~~disclosure to the subject.

30 In another aspect, the present disclosure provides a use of a cell, a population of cells, or a population of gene edited cells in the manufacture of a medicament for treating a RAG-deficient immunodeficiency in a subject.

35 In another aspect, the present ~~invention~~disclosure provides a method of treating immunodeficiency in a subject in need thereof comprising administering a cell, a population of cells, or a population of gene edited cells of the present ~~invention~~disclosure to the subject.

In another aspect, the present ~~invention~~disclosure provides a cell, a population of cells, or a population of gene edited cells of the present ~~invention~~disclosure for use as a medicament.

In another aspect, the present ~~invention~~disclosure provides a cell, a population of cells, or a population of gene edited cells of the present ~~invention~~disclosure for use in treating immunodeficiency in a subject.

## DESCRIPTION OF DRAWINGS

### 5 **Figure 1. Generation of NALM6 Cas9 and K562 Cas9 cell lines**

**A)** Schematic representation of the gene correction approach; **B)** Schematic representation of the protocol for generation of K562 Cas9 and NALM6 Cas9 cell lines; **C)** Vector Copy Number (VCN) of the integrated Cas9 containing cassette measured by ddPCR, telomerase was used as normalizer; **D)** Cas9 expression for scaling doses of doxycycline measured by qPCR in NALM6  
10 Cas9 (left panel) and K562 Cas9 (right panel) cell lines, represented as fold change Vs actin.

### **Figure 2. Selection of the best performing gRNA**

**A)** Schematic representation of the intronic and exonic loci targeted by the different gRNA tested; **B)** Schematic representation of the experimental protocol; **C)** Percentages of NHEJ induced indels in K562 Cas9 treated with different doses of plasmids encoding for different guides, 7 days after  
15 transfection, n=1; **D)** Percentages of NHEJ induced indels in NALM6 Cas9 treated with different doses of plasmids encoding for guides 3, 7 and 9, 7 days after transfection, n=1; **E)** Percentages of NHEJ induced indels in NALM6 Cas9 treated with different doses of guides 3 and 9 *in vitro* preassembled RNPs 7 days after transfection, n=1.

### **Figure 3. Donor DNA optimization**

**A)** RAG1 gene expression measured by RT-qPCR, represented as fold change vs RAG1 expression in 293T cell line, actin was used as normalizer; **B)** Schematic representation of different SA\_GFP DNA donor tested; **C)** Schematic representation of the splicing mechanism with SA\_GFP\_SD donor; **D)** Percentage of targeted cells measured by flow cytometry as GFP+ cells,  
20 7 days after transfection; **E)** GFP expression levels measured as Mean Fluorescence Intensity (MFI) gating on GFP+ events; **F)** Representative FlowJo plots; One-way ANOVA, Geisser-Greenhouse correction for multiple comparison, n=3. *P* values: \*<0.05; \*\*<0.005; \*\*\*<0.0005; \*\*\*\*<0.0001. Mean±SD are shown.

### **Figure 4. Off-target analysis**

**A)** Table shows the top 10 off-target sites predicted by in silico COSMID tool for guide 9. The off-  
30 target sequence, type of PAM, score, number of mismatches and chromosomal position are shown. **B-C)** Cutting efficiency measured as percentage of NHEJ (D) and dsDNA tag

## CLAIMS

1. An isolated polynucleotide comprising from 5' to 3': a first homology region, a splice acceptor sequence, a nucleotide sequence encoding a RAG1 polypeptide, and a second homology region.
2. The isolated polynucleotide according to claim 1, wherein:
  - (i) the first homology region is homologous to a first region of the RAG1 intron 1 and the second homology region is homologous to a second region of the RAG1 intron 1;  
or
  - (ii) the first homology region is homologous to a first region of the RAG1 intron 1 or the RAG1 exon 2 and the second homology region is homologous to a second region of the RAG1 exon 2.
3. The isolated polynucleotide according to claim 1 or claim 2, wherein the first homology region is homologous to a first region of the RAG1 intron 1 and the second homology region is homologous to a second region of the RAG1 intron 1.
4. The isolated polynucleotide according to any preceding claim, wherein:
  - (i) the first homology region is homologous to a region upstream of chr 11: 36569295 and the second homology region is homologous to a region downstream of chr 11: 36569298;
  - (ii) the first homology region is homologous to a region upstream of chr 11: 36573790 and the second homology region is homologous to a region downstream of chr 11: 36573793;
  - (iii) the first homology region is homologous to a region upstream of chr 11: 36573641 and the second homology region is homologous to a region downstream of chr 11: 36573644;
  - (iv) the first homology region is homologous to a region upstream of chr 11: 36573351 and the second homology region is homologous to a region downstream of chr 11: 36573354;
  - (v) the first homology region is homologous to a region upstream of chr 11: 36569080 and the second homology region is homologous to a region downstream of chr 11: 36569083;

(vi) the first homology region is homologous to a region upstream of chr 11: 36572472 and the second homology region is homologous to a region downstream of chr 11: 36572475;

(vii) the first homology region is homologous to a region upstream of chr 11: 36571458 and the second homology region is homologous to a region downstream of chr 11: 36571461;

(viii) the first homology region is homologous to a region upstream of chr 11: 36571366 and the second homology region is homologous to a region downstream of chr 11: 36571369;

(ix) the first homology region is homologous to a region upstream of chr 11: 36572859 and the second homology region is homologous to a region downstream of chr 11: 36572862;

(x) the first homology region is homologous to a region upstream of chr 11: 36571457 and the second homology region is homologous to a region downstream of chr 11: 36571460;

(xi) the first homology region is homologous to a region upstream of chr 11: 36569351 and the second homology region is homologous to a region downstream of chr 11: 36569354; or

(xii) the first homology region is homologous to a region upstream of chr 11: 36572375 and the second homology region is homologous to a region downstream of chr 11: 36572378.

5. The isolated polynucleotide according to any preceding claim, wherein:

(i) the first homology region is homologous to a region upstream of chr 11: 36569295 and the second homology region is homologous to a region downstream of chr 11: 36569298;

(ii) the first homology region is homologous to a region upstream of chr 11: 36573351 and the second homology region is homologous to a region downstream of chr 11: 36573354; or

(iii) the first homology region is homologous to a region upstream of chr 11: 36571366 and the second homology region is homologous to a region downstream of chr 11: 36571369;

preferably wherein the first homology region is homologous to a region upstream of chr 11: 36569295 and the second homology region is homologous to a region downstream of chr 11: 36569298.

6. The isolated polynucleotide according to any preceding claim, wherein the first homology region is homologous to a region comprising chr 11: 36569245-chr 11: 36569294 and/or the second homology region is homologous to a region comprising chr 11: 36569299-chr 11: 36569348.

7. The isolated polynucleotide according to any preceding claim, wherein the 3' terminal sequence of the first homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 7 and/or the 5' terminal sequence of the second homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 19.

8. The isolated polynucleotide according to any preceding claim, wherein the first homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 31, or a fragment thereof and/or the second homology region comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 32, or a fragment thereof.

9. The isolated polynucleotide according to any preceding claim, wherein the first and second homology regions are each 50-1000bp in length, 100-500 bp in length, or 200-400 bp in length.

10. The isolated polynucleotide according to any preceding claim, wherein the nucleotide sequence encoding a RAG1 polypeptide comprises or consists of a nucleotide sequence encoding an amino acid sequence that has at least 70% identity to SEQ ID NO: 4 or SEQ ID NO: 5.

11. The isolated polynucleotide according to any preceding claim, wherein the nucleotide sequence encoding a RAG1 polypeptide comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 6.

12. The isolated polynucleotide according to any preceding claim, wherein the splice acceptor site comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 33.

13. The isolated polynucleotide according to any preceding claim, wherein the nucleotide sequence encoding a RAG1 polypeptide is operably linked to a polyadenylation sequence, optionally wherein the polyadenylation sequence is a bGH polyadenylation sequence.
14. The isolated polynucleotide according to any preceding claim, wherein the nucleotide sequence encoding a RAG1 polypeptide is operably linked to a polyadenylation sequence comprising or consisting of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 35.
15. The isolated polynucleotide according to any preceding claim, wherein the nucleotide sequence encoding a RAG1 polypeptide is operably linked a Kozak sequence, optionally wherein the Kozak sequence comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 36.
16. The isolated polynucleotide according to any preceding claim, wherein the polynucleotide comprises or consists of a nucleotide sequence that has at least 70% identity to SEQ ID NO: 39.
17. A vector comprising the polynucleotide according to any preceding claim.
18. The vector according to claim 17, wherein the vector is a viral vector, optionally an adeno-associated viral (AAV) vector such as an AAV6 vector.
19. A guide RNA comprising or consisting of a nucleotide sequence that has at least 90% identity to any of SEQ ID NOs: 41-52 or 53-55, optionally wherein the guide RNA comprises or consists of a nucleotide sequence that has at least 90% identity to SEQ ID NO: 41 or 53 (preferably SEQ ID NO: 41).
20. The guide RNA according to claim 19, wherein from one to five of the terminal nucleotides at 5' end and/or 3' end of the guide RNA are chemically modified to enhance stability, optionally wherein three terminal nucleotides at 5' end and/or 3' end if the guide RNA are chemically modified to enhance stability, optionally wherein the chemical modification is modification with 2'-O-methyl 3'phosphorothioate.
21. A kit, a composition, or a gene-editing system, comprising the polynucleotide according to any one of claims 1 to 16 or the vector according to any one of claims 17 or 18.
22. The kit, composition, gene-editing system according to claim 21, wherein the kit, composition, or gene-editing system further comprises a guide RNA according to claim 19 or claim 20.

23. The kit, composition, or gene-editing system, according to claim 21 or claim 22, wherein the kit, composition, or gene-editing system, further comprises a RNA-guided nuclease, optionally wherein the RNA-guided nuclease is a Cas9 endonuclease.

24. Use of the isolated polynucleotide according to any one of claims 1 to 16, the vector according to any one of claims 17 or 18, the guide RNA according to any one of claims 19 or 20, or the kit, composition, or gene-editing system according to any one of claims 21 to 23, for gene editing a cell or a population of cells.

25. An isolated genome comprising the polynucleotide according to any one of claims 1 to 16.

26. An isolated cell comprising the polynucleotide according to any one of claims 1 to 16 or the genome according to claim 25.

27. The isolated cell according to claim 26, wherein the cell is a hematopoietic stem cell (HSC), a hematopoietic progenitor cell (HPC), or a lymphoid progenitor cell (LPC).

28. The isolated cell according to claim 26 or claim 27, wherein the cell is a CD34+ cell.

29. A population of cells comprising one or more isolated cells according to any one of claims 26 to 28.

30. The population of cells according to claim 29, wherein at least 50% of the population of cells are CD34+ cells.

31. The population of cells according to claim 29 or claim 30, wherein at least 20% of the population of cells are CD34+ cells comprising the genome according to claim 25.

32. A method of gene editing a population of cells comprising:

(a) providing a population of cells; and

(b) delivering an RNA-guided nuclease, a guide RNA according to claim 19 or claim 20, and a vector according to claim 17 or claim 18, to the population of cells to obtain a population of gene-edited cells.

33. Use of a population of gene-edited cells in the manufacture of a medicament for A  
~~method of treating a RAG-deficient immunodeficiency in a subject, wherein the use~~  
~~comprises~~ing:

~~(a) providing a population of cells;~~

~~(b) delivering an RNA-guided nuclease, a guide RNA according to claim 19 or claim 20, and a vector according to claim 17 or claim 18, to be delivered to a~~ population of cells to obtain ~~the~~ a population of gene-edited cells, ~~and wherein~~

~~(c) administering the population of gene-edited cells is to be administered to the subject.~~

34. The method according to claim 32, or the use according to claim 33, wherein the population of cells comprises or consists of HSCs, HPCs, and/or LPCs and/or wherein the population of cells comprises or consists of CD34+ cells.

35. The method according to any one of claims 32 and 34, or the use according to any one of claims 33 to and 34, wherein the population of cells is pre-activated, optionally wherein the population of cells is cultured with one or more cytokines selected from: one or more early acting cytokines such as TPO, IL-6, IL-3, SCF, FLT3-L; one or more transduction enhancers such as PGE2; and one or more expansion enhancers such as UM171, UM729, SR1.

36. The method according to any one of claims 32 and 34 to 35, or the use according to any one of claims 33 to 35, wherein the RNA-guided nuclease and/or guide RNA is delivered prior to the vector and/or simultaneously with the vector.

37. The method according to any one of claims 32 and 34 to 36, or the use according to any one of claims 33 to 36, wherein the RNA-guided nuclease is Cas9, optionally wherein the Cas9 and the guide RNA are delivered preassembled as Cas9 RNPs.

38. The method according to any one of claims 32 and 34 to 37, or the use according to any one of claims 33 to 37, wherein the method further comprises delivering a p53 inhibitor and/or a HDR enhancer, optionally wherein the p53 inhibitor and/or a HDR enhancer is delivered simultaneously with the RNA-guided nuclease and/or guide RNA.

39. The method according to any one of claims 32 and 34 to 38, or the use according to any one of claims 33 to 38, wherein the population of gene-edited cells is defined according to any one of claims 29 to 31.

40. A population of gene-edited cells obtainable by the method according to any one of claims 32 and 34 to 39.

~~41. A method of treating a RAG-deficient immunodeficiency comprising administering the isolated cell according to any one of claims 26 to 28, the population of cells according to any~~

~~one of claims 29 to 31, or the population of gene-edited cells according to claim 40, to a subject in need thereof.~~

~~4241. Use of T~~the isolated cell according to any one of claims 26 to 28, the population of cells according to any one of claims 29 to 31, or the population of gene-edited cells according to claim 40, in the manufacture of a medicament for use in treating a RAG-deficient immunodeficiency in a subject.

~~4342. The method use according to claim 41, or the isolated cell, population of cells, or population of gene-edited cells for use according to claim 42,~~ wherein the RAG-deficient immunodeficiency is T- B- severe combined immunodeficiency (SCID), Omenn syndrome, atypical SCID or combined immunodeficiency with granuloma/autoimmunity (CID-G/AI).

~~4443. The method use according to claim 41 or claim 42 or claim 43, or the isolated cell, population of cells, or population of gene-edited cells for use according to claim 42 or claim 43,~~ wherein the subject has a RAG1 deficiency.

~~4544. The method use according to any one of claims 41, 42, or 43, 43, or 44, or the isolated cell, population of cells, or population of gene-edited cells for use according to any one of claims 42 to 44,~~ wherein the subject has a mutation in the RAG1 gene, optionally in RAG1 exon 2.