

CLAIMS

1. Use of (i) a first polynucleotide, a first expression cassette, a first vector, or a first rAAV particle comprising a nucleotide sequence encoding a human insulin (Ins) protein, wherein the nucleotide sequence is: (a) at least 90%, 95%, 99%, or 100% identical to any one of nucleic acids 73-330 of any of SEQ ID NOs: 43-57, 110-116, 150-151, 154-155 or 157-159, nucleic acids 88-345 of any of SEQ ID NOs: 117-122, 152 or 156, or nucleic acids 79-336 of SEQ ID NO: 153, wherein the human Ins protein comprises a signal peptide, wherein the signal peptide is an IL-6 signal sequence; and
(ii) a second polynucleotide, a second expression cassette, a second vector, or a second rAAV particle comprising a nucleotide sequence encoding a human glucokinase (Gck) protein, wherein the nucleotide sequence is at least 95%, 99%, or 100% identical to a sequence selected from any of (a) nucleic acids 1-1398 of any of SEQ ID NO: 61-80 and 162; or (b) SEQ ID NO: 61-80 and 162 in the manufacture of a medicament for treating or ameliorating the symptoms associated with diabetes in a subject, wherein the administration of the medicament is intramucular.
2. The use of claim 1, wherein the encoded human Ins protein comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, amino acids 25-110 of SEQ ID NO: 145.
3. The use of any one of claims 1 or 2, wherein the polynucleotide, the expression cassette, the vector, or the rAAV particle further comprises a promoter operably linked to the nucleotide sequence encoding the human Ins protein.
4. The use of claim 3, wherein the promoter is a constitutive promoter.
5. The use of claim 3 or 4, wherein the promoter is a CMV promoter.
6. The use of any one of claims 1-5, wherein the vector comprising the nucleotide sequence encoding the human Ins protein is an adeno-associated virus (AAV) vector.
7. The use of any one of claims 1-6, wherein the encoded human Gck protein comprises the amino acid sequence of SEQ ID NO: 82.

8. The use of any one of claims 1-7, wherein the polynucleotide, the expression cassette, the vector, or the rAAV particle comprising the nucleotide sequence encoding the human Gck comprises a sequence at least 90%, 95%, 99%, or 100% identical to a sequence selected from any of SEQ ID NOs: 20-39, 89-96, and 163-164 or any combination thereof.
9. The use of any one of claims 1-8, wherein the polynucleotide, the expression cassette, the vector, or the rAAV particle further comprises a promoter operably linked to the nucleotide sequence encoding the human Gck protein.
10. The use of claim 9, wherein the promoter is a constitutive promoter.
11. The use of claim 9 or 10, wherein the promoter is a CMV promoter.
12. The use of any one of claims 1-11, wherein (i) the polynucleotide, the expression cassette, the vector, or the rAAV particle comprising the nucleotide sequence encoding the human Ins protein is at least 97%, 99%, or 100% identical to SEQ ID NO: 121; and (ii) the polynucleotide, an expression cassette, a vector, or a rAAV particle comprising the nucleotide sequence encoding the human Gck protein is at least 95%, 99%, or 100% identical to SEQ ID NO: 68.
13. The use of any one of claims 1-12, wherein the AAV serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVRH8, AAVrh9, AAV9, AAVrh10, AAV10, AAVRH10, AAV11, AAV12.
14. The use of any one of claims 1-13, wherein the AAV serotype of the AAV particles is AAV1.
15. The use of any one of claims 1-14, wherein the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM).
16. The use of any one of claims 1-15, wherein the first polynucleotide, the first expression cassette, the first vector, or the first rAAV particle is administered simultaneously or sequentially with the second polynucleotide, the second expression cassette, the second vector, or the second rAAV particle.
17. The use of any one of claims 1-16, wherein (i) glycated blood hemoglobin (HbA1c) levels are reduced and/or regulated in a subject after administration; (ii) circulating ketones are

reduced in a subject after administration, (iii) triglycerides are reduced in a subject after administration, or (iv) any combination thereof.

18. A polynucleotide encoding a human insulin (Ins) protein comprising (i) a nucleotide sequence encoding an IL-6 signal peptide, and (ii) a nucleotide sequence encoding a proinsulin polypeptide comprising an amino acid modification at a position selected from amino acid B10, B28, and/or B29 of the human insulin B-chain, C1 and/or C32 of the human insulin C-chain, or any combination thereof relative to the corresponding amino acid position in wild-type proinsulin.

19. A polynucleotide comprising a nucleic acid encoding a human insulin (Ins) protein, wherein the nucleic acid encoding the human Ins protein comprises a nucleotide sequence at least 90%, 95%, 99%, or 100% identical to any one of nucleic acids 73-330 of any of SEQ ID NOs: 43-57, 110-116, 150-151, 154-155 or 157-159, nucleic acids 88-345 of any of SEQ ID NOs: 117-122, 152 or 156, or nucleic acids 79-336 of SEQ ID NO: 153, wherein the human Ins protein further comprises an IL-6 signal sequence.

20. The polynucleotide of claim 18, wherein the encoded human Ins protein comprises the amino acid sequence of any of amino acids 25-110 of SEQ ID NO: 41, amino acids 25-110 of SEQ ID NO: 144, amino acids 25-110 of SEQ ID NO: 145.

21. The polynucleotide of any of claims 18-20, wherein the human Ins protein further comprises a cleavage site.

22. The polynucleotide of any one of claims 18-21, wherein the nucleic acid encoding the human Ins protein further comprises a 5' UTR comprising a nucleotide sequence at least 90%, at least 95%, at least 99%, or 100% identical to nucleic acids 5-329 of SEQ ID NO: 42.

23. The polynucleotide of any of claims 18-22, wherein the nucleic acid further comprises a 5' UTR comprising a nucleotide sequence at least 90%, 95%, 99%, or 100% identical to SEQ ID NO: 42, SEQ ID NO: 83, SEQ ID NO: 146, or SEQ ID NO: 148.

24. The polynucleotide of any one of claims 18-23, wherein the nucleic acid encoding the human Ins protein further comprises a 3' UTR comprising a nucleotide sequence at least 90%, at

least 95%, at least 99%, or 100% identical to SEQ ID NO: 60, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, or SEQ ID NO: 101, or SEQ ID NO: 149.

25. The polynucleotide of any one of claims 18-24, wherein the 3' UTR further comprises a restriction site selected from the group consisting of BamHI, EcoRI, NdeI, EcoRV, SpeI, XbaI, NheI, VspI, NsiI, ScaI, KpnI, SspI, and PacI.

26. The polynucleotide of any one of claims 18-25, which comprises at least two open reading frames (ORFs) that encode human Ins protein.

27. The polynucleotide of claim 26, wherein the at least two ORFs are operably linked by an IRES sequence.

28. The polynucleotide of claim 27, wherein the IRES sequence comprises a nucleotide sequence at least 90%, at least 95%, at least 99%, or 100% identical to SEQ ID NO: 142 or SEQ ID NO: 143.

29. The use of polynucleotide of any one of claims 1-28, wherein the nucleic acid encoding the human Ins protein comprises a nucleotide sequence at least 90%, at least 95%, at least 99%, or 100% identical to any of SEQ ID NOs: 1-16, or any combination thereof.

30. The use of polynucleotide of any one of claims 1-28, wherein the nucleic acid encoding the human Ins protein comprises a nucleotide sequence at least 90%, at least 95%, at least 99%, or 100% identical to any of SEQ ID NOs: 84-88, or any combination thereof.

31. The use of polynucleotide of any one of claims 1-28, wherein the nucleic acid encoding the human Ins protein comprises a nucleotide sequence at least 90%, at least 95%, at least 99%, or 100% identical to any of SEQ ID NOs: 123-126, 128-141, and 160-161, or any combination thereof.

32. An expression cassette comprising the polynucleotide of any one of claims 18-31 and a promoter operably linked to the nucleic acid sequence.

33. The expression cassette of claim 32, wherein the promoter is a constitutive promoter.

34. The expression cassette of claim 33, wherein the promoter is a CMV promoter.

35. The polynucleotide or expression cassette of any one of claims 18-34, wherein the nucleic acid is operably linked to a polyadenylation (polyA) element.
36. A vector comprising the polynucleotide or expression cassette of any of claims 18-35.
37. The vector of claim 36, wherein the vector is a viral vector, a non-viral vector, a plasmid, a lipid, or a lysosome.
38. The vector of claim 37, wherein the vector is an adeno-associated virus (AAV) vector or a Lentivirus vector.
39. A recombinant AAV (rAAV) particle, comprising an AAV capsid and a vector genome comprising the polynucleotide or the expression cassette of any of claims 18-35.
40. The vector or rAAV particle of any one of claims 36-38, wherein the AAV serotype is selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAVRH8, AAVrh9, AAV9, AAVrh10, AAV10, AAVRH10, AAV11, AAV12.
41. A host cell comprising the polynucleotide, the expression cassette, the vector, or the rAAV particle of any one of claims 18-40.
42. The host cell of claim 41, wherein the host cell is a mammalian cell.
43. A composition comprising the polynucleotide, the expression cassette, the vector, or rAAV particle of any of claims 18-40.
44. A method of producing human Ins protein in a cell, comprising contacting and/or transforming the cell with the polynucleotide, the expression cassette, the vector, rAAV particle, or composition of any of claims 18-40 and 43, thereby producing the human Ins protein in the cell.
45. The polynucleotide, the expression cassette, the vector, rAAV particle, or composition of any of claims 18-40 and 43 for use in the treatment of diabetes, wherein the polynucleotide, expression cassette, vector, rAAV particle, or composition is for intramuscular administration.
46. The polynucleotide, expression cassette, vector, rAAV particle, or composition of claim 45, wherein the diabetes is diabetes mellitus type 1 (T1DM) or diabetes mellitus type 2 (T2DM).

47. The polynucleotide, expression cassette, vector, rAAV particle, or composition of claim 45 or 46, wherein (i) glycated blood hemoglobin (HbA1c) levels are reduced and/or regulated in a subject after administration; (ii) circulating ketones are reduced in a subject after administration, (iii) triglycerides are reduced in a subject after administration, or (iv) any combination thereof.