CLAIMS

~~WHAT IS CLAIMED IS:~~

1. An isolated transgenic porcine islet cell, wherein said cell:

   is substantially free of enzymatic activity of at least one glycosyltransferase enzyme, wherein said glycosyltransferase enzyme is GGTA, B4GALNT2, or CMAH;

   expresses at least two polypeptide sequences derived from a non-porcine mammalian species, wherein said at least two polypeptide sequences comprise at least two of CD46, CD55, CD59, THBD, TFPI, CD39, B2M, HLAE, CD47, A20, PD-L1, FASL, or HO-1; and

   exhibits one or more of the following: reduced toxicity from complement derived from said non-porcine mammalian species, reduced induction of activated protein C coagulation derived from said non-porcine mammalian species, reduced induction of thrombin-antithrombin complex derived from said non-porcine mammalian species, or reduced toxicity from NK cells derived from said non-porcine species.

2. The transgenic porcine islet cell of claim 1, wherein said cell is substantially free of enzymatic activity of at least two, or all three glycosyltransferase enzymes selected from GGTA, B4GALNT2, and CMAH.

3. The transgenic porcine islet cell of claim 1 or 2, wherein said cell expresses at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or all of: CD46, CD55, CD59, THBD, TFPI, CD39, B2M, HLAE, CD47, A20, PD-L1, FASL, or HO-1.

4. An isolated transgenic porcine islet cell, wherein said islet cell:

   (a) is substantially free of enzymatic activity of at least two glycosyltransferase enzymes, wherein said glycosyltransferase enzymes comprise at least two of GGTA, B4GALNT2, or CMAH;

   (b) expresses a polypeptide sequence derived from a non-porcine mammalian species, wherein said polypeptide sequence is CD46, CD55, CD59, THBD, TFPI, CD39, B2M, HLAE, CD47, A20, PD-L1, FASL, or HO-1; and

   (c) exhibits reduced toxicity from complement derived from said non-porcine mammalian species, reduced induction of activated protein C coagulation derived from said non-porcine species, reduced induction of thrombin-antithrombin complex

derived from said non-porcine species, or reduced toxicity from NK T-cells derived from said non-porcine species.

5. The transgenic porcine islet cell of claim 4, wherein said cell is substantially free of enzymatic activity of GGTA, B4GALNT2, and CMAH.
6. The transgenic porcine islet cell of claim 4 or 5, wherein said cell expresses at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, at least eleven, at least twelve, or all of CD46, CD55, CD59, THBD, TFPI, CD39, B2M, HLAE, CD47, A20, PD-L1, FASL, or HO-1.
7. The transgenic porcine islet cell of any one of claims 1-6, expressing CD46, CD55, CD59, CD39, B2M, HLAE, and CD47.
8. The transgenic porcine islet cell of any one of claims 1-6, wherein said cell is substantially free of expression of said glycosyltransferase enzyme or enzymes.
9. The transgenic porcine islet cell of claim 8, wherein said cell comprises a frameshift mutation in said glycosyltransferase enzyme or enzymes resulting in premature termination of translation, thereby ablating activity of said glycosyltransferase enzyme.
10. The transgenic porcine islet cell of any one of claims 1-9, wherein a nucleic acid sequence or sequences encoding said polypeptide sequence or sequences derived from non-porcine mammalian species are inserted within non-orthologous loci of the porcine ortholog.
11. The transgenic porcine islet cell of any one of claims 1-10, wherein a nucleic acid sequence or sequences encoding said polypeptide sequence or sequences derived from non-porcine mammalian species are operably linked to non-orthologous promoters of the porcine ortholog.
12. The transgenic porcine islet cell of claim 11, wherein said non-orthologous promoters are non-porcine promoters.
13. The transgenic porcine islet cell of any one of claims 1-12, wherein said islet cell is derived by disaggregation of a porcine pancreas.
14. The transgenic porcine islet cell of any one of claims 1-13, wherein said islet cell is an alpha cell, a beta cell, a delta cell, an epsilon cell, a Pancreatic polypeptide (PP) cell, or any combination thereof.
15. The transgenic porcine islet cell of any one of claims 1-14, wherein said non-porcine mammalian species is a primate species.
16. The transgenic porcine islet cell of any one of claims 1-15, wherein said cell exhibits survival greater than 8 days when transplanted into said non-porcine mammalian species.

17. The transgenic porcine cell of any one of claims 1-16, wherein said cell exhibits a reduced IBMIR to PBMCs isolated from said non-porcine mammalian species.
18. A composition comprising a therapeutically effective amount of isolated porcine islet cells according to any one of claims 1-17.
19. The composition of claim 18, wherein said isotonic buffered solution further comprises heparin or a TNF-alpha inhibitor.
20. The composition of claim 18 or 19, comprising at least about 12% to about 25% beta cells or at least about 15% to about 30% alpha cells.
21. The composition of any one of claims 18-20, wherein said composition is prepared according to any one of claims 35-42.
22. Use of at least one isolated transgenic porcine islet cell according to any one of claims 1-17 or a composition according to any one of claims 18-21 in preparation of a medicament for ~~A method of~~ treating an insulin resistant or deficient condition in a non-porcine mammal~~ in need thereof, comprising administering a therapeutically effective dose of isolated transgenic porcine islet cells according to any one of claims 1-17 or a composition according to any one of claims 18-21 to said mammal~~.
23. The ~~method~~ use of claim 22, comprising ~~centrally administering~~ said cells to be centrally administered via an internal jugular vein or a hepatic portal vein of said mammal.
24. The ~~method~~ use of any one of claims 22-23, wherein said insulin resistant condition comprises type 1 diabetes mellitus.
25. The ~~method~~ use of any one of claims 22-23, wherein said insulin resistant condition comprises type 2 diabetes mellitus.
26. The ~~method~~ use of any one of claims 22-25, wherein said non-porcine mammal is to be treated with ~~has received~~ an induction regimen comprising therapeutically effective doses of anti-thymocyte globulin, anti-CD40 antibody, anti-CD20 antibody, a rapalog, a calcineurin inhibitor, ganciclovir or a prodrug thereof, an antihistamine, and a corticosteroid prior to the administration of~~administering~~ said transgenic porcine islet cell or said composition.
27. The ~~method~~ use of any one of claims 22-26, comprising ~~administering~~ therapeutically effective doses of anti-CD40 antibody, a rapalog, a calcineurin inhibitor, and ganciclovir or a prodrug thereof to be administered following administration of said transgenic porcine islet cell or said composition.

28. The ~~method~~ use of any one of claims 22-27, comprising ~~administering~~ therapeutically effective doses of an intermediate- or long-acting insulin analog, insulin glargine, insulin detemir, or NPH insulin to be administered following administration of said transgenic porcine islet cell or said composition.
29. The ~~method~~ use of any one of claims 22-28, wherein said therapeutically effective dose is at least 5,000 IEQ per kg of non-porcine mammal body weight.
30. An isolated porcine islet comprising an isolated porcine islet cell according to any one of claims 1-17.
31. An isolated porcine pancreatic organoid comprising an isolated porcine islet cell according to any one of claims 1-17.
32. The isolated porcine islet or isolated porcine pancreatic organoid of claim 30 or 31, wherein said islet or organoid is substantially free of pancreatic exocrine cells.
33. The isolated porcine pancreatic organoid of claim 31 or 32; wherein said pancreatic organoid is prepared by:
    (a) isolating a pancreas from a neonatal porcine animal on neonatal day 7 or earlier; and
    (b) subjecting said pancreas to mechanical or enzymatic digestion to generate organoid fragments, and optionally:
    (c) purifying organoid fragments of step (b) by ficoll gradient sedimentation.
34. An isolated porcine pancreas comprising a porcine islet cell according to any one of claims 1-17.
35. A method of improving yield of islets from a porcine donor prior to transplantation to a non-porcine mammalian recipient, comprising
    (a) providing isolated pancreatic organoids from a neonatal porcine animal that have been subjected to a purification procedure;
    (b) culturing said organoids in the presence of an effective concentration of a caspase inhibitor for at least 90 minutes following said purification; and
    (c) continuing culture in the presence of an effective concentration of a corticosteroid for at least 7 days.
36. The method of claim 35; wherein said purification procedure comprises:
    (a) providing an isolated~~isolating a~~ pancreas from a transgenic neonatal porcine animal on neonatal day 7 or earlier; and

(b) subjecting said pancreas to mechanical or enzymatic digestion to generate organoid fragments, and optionally:

(c) purifying organoid fragments from said digested pancreas by ficoll gradient sedimentation.

37. The method of claim 35 or 36, wherein said neonatal porcine animal is a transgenic pig comprising at least one porcine cell according to any one of claims 1-17.

38. Use of at least one porcine cell according to any one of claims 1-17 in the preparation of a composition for improving yield of islets from a porcine donor prior to transplantation to a non-porcine mammalian recipient, comprising:

(a) providing isolated pancreatic organoids from a neonatal porcine animal that have been subjected to a purification procedure;

(b) culturing said organoids in the presence of an effective concentration of a caspase inhibitor for at least 90 minutes following said purification; and

(c) continuing culture in the presence of an effective concentration of a corticosteroid for at least 7 days.

39. The use of claim 38, wherein said purification procedure comprises:

(a) providing an isolated pancreas from a transgenic neonatal porcine animal on neonatal day 7 or earlier; and

(b) subjecting said pancreas to mechanical or enzymatic digestion to generate organoid fragments, and optionally:

(c) purifying organoid fragments from said digested pancreas by ficoll gradient sedimentation.

~~38~~40. The method of any one of claims 35-37 or the use of claims 38-39, wherein said caspase inhibitor is Z-VAD-FMK.

~~39~~41. The method of any one of claims 35-~~38~~37 and 40, or the use of any one of claims 38-40, wherein said corticosteroid is methylprednisolone.

~~40~~42. The method of any one of claims 35-~~39~~37 and 40-41, or the use of any one of claims 38-41, wherein said pancreatic organoids are cultured in the presence of IBMX, a phosphodiesterase inhibitor, or an adenosine receptor antagonist.

~~41~~43. The method of any one of claims 35-~~40~~37 and 40-42, or the use of any one of claims 38-42, wherein said pancreatic organoids are cultured in the presence of nicotinamide or a metabolically acceptable analog thereof.

4244. ~~A method of treating an insulin resistant or deficient condition in a non-procine mammal in need thereof, comprising~~ Use of transplanting organoids prepared according to the method of any one of claims 35-~~41~~ 37 and 39-43 for the preparation of a composition for the treatment of an insulin resistant or deficient condition in a non-porcine mammal, wherein said composition is to be transplanted into said non-porcine mammalian mammal when said organoids meet any of the following criteria

(a) endotoxin less than about 5EU/kg;

(b) negative gram stain;

(c) viability greater than about 70%; or

(d) islet concentration greater than or equal to about 20,000 IEQ/mL of total settled volume.