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(54) Title: COMPOSITIONS AND METHODS FOR INTERNALIZING ENZYMES

(57) Abstract: Compositions and methods for treating lysosomal storage diseases are disclosed. Biotherapeutic complexes containing an internalization effector binding domain and a lysosomal replacement enzyme activity are disclosed. The biotherapeutic complexes are capable of entering cells, segregating to the lysosome, and delivering the replacement enzyme activity to the lysosome.

AMENDED CLAIMS
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CLAIMS

1. A composition comprising a lysosomal enzyme linked directly or by a single linker to an antigen-binding protein that binds a membrane protein that undergoes endocytosis.

2. The composition of claim 1, wherein the membrane protein localizes to the lysosomal membrane.

3. The composition of claim 1, wherein the lysosomal enzyme is selected from the group consisting of α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, saposin-C activator, ceramidase, sphingomyelinase, β-hexosaminidase, GM2 activator, GM3 synthase, arylsulfatase, sphingolipid activator, α-iduronidase, iduronidase-2-sulfatase, heparin N-sulfatase, N-acetyl-α-glucosaminidase, α-glucosamide N-acetyltransferase, N-acetylglucosamine-6-sulfatase, N-acetylgalactosamine-6-sulfate sulfatase, N-acetylgalactosamine-4-sulfatase, β-glucuronidase, and hyaluronidase.

4. The composition of any one of claims 1-3, wherein the antigen-binding protein is selected from the group consisting of a receptor-fusion molecule, a trap molecule, a receptor-Fc fusion molecule, an antibody, an Fab fragment, an F(ab')2 fragment, an Fd fragment, an Fv fragment, a single-chain Fv (scFv) molecule, a dAb fragment, an isolated complementarity determining region (CDR), a CDR3 peptide, a constrained FR3-CDR3-FR4 peptide, a domain-specific antibody, a single domain antibody, a domain-deleted antibody, a chimeric antibody, a CDR-grafted antibody, a

diabody, a triabody, a tetrabody, a minibody, a nanobody, a monovalent nanobody, a bivalent nanobody, a small modular immunopharmaceutical (SMIP), a camelid antibody (VHH heavy chain homodimeric anibody), and a shark variable IgNAR domain.

5. The composition of any one of claims 1-4, wherein the membrane protein is selected from the group consisting of CD63, MHC-I, Kremen-1, Kremen-2, LRP5, LRP6, LRP8, transferrin receptor, LDL-receptor, LDL-related protein 1 receptor, ASGR1, ASGR2, amyloid precursor protein-like protein-2 (APLP2), apelin receptor (APLNR), PRLR (prolactin receptor), MAL (Myelin And Lymphocyte protein, a.k.a. VIP17), IGF2R, vacuolar-type H+ ATPase, diphtheria toxin receptor, folate receptor, glutamate receptors, glutathione receptor, leptin receptors, scavenger receptors, SCARA1-5, SCARB1-3, CD36, CDH16 (Cadheri-16), CLDN16 (Claudn-16), KL (Klotho), PTH1R (parathyroid hormone receptor), SLC22A13 (Solute carrier family 22 member 13), SLC5A2 (Sodium/glucose cotransporter 2), UMOD (Uromodulin), BMPR1A (Bone morphogenetic protein receptor 1A), m-cadherin, CD9, MuSK (muscle-specific kinase), LGR4/GPR48 (G protein-coupled receptor 48), cholinergic receptor (nicotinic) alpha 1, CDH15 (Cadheri-15), ITGA7 (Integrin alpha-7), CACNG1 (L-type calcium channel subunit gamma-1), CACNAls (L-type calcium channel subunit alpha-15), CACNG6 (L-type calcium channel subunit gamma-6), SCN1B (Sodium channel subunit beta-1), CHRNA1 (ACh receptor subunit alpha), CHRND (ACh receptor subunit delta), LRRC14B (Leucine-rich repeat-containing protein 14B), and POPDC3 (Popeye domain-containing protein 3).

6. The composition of any one of claims 1-5, wherein the lysosomal enzyme is directly linked to the antigen-binding protein.

7. The composition of claim 6, wherein the antigen-binding protein comprises an antibody, and the enzyme is covalently linked to the C-terminus of the heavy chain of the antibody.

8. The composition of any one of claims 1-5, wherein the lysosomal enzyme is linked by a single linker to the antigen-binding protein.

9. The composition of claim 8, wherein the antigen-binding protein comprises a half-antibody, the lysosomal enzyme is covalently linked to an immunoglobulin Fc-domain, and the Fc-domain that is covalently linked to the enzyme associates with the Fc-domain of the antigen-binding protein.

10. The composition of claim 8, wherein the linker is a cleavable linker.

11. The composition of any one of claims 1-10, wherein the lysosomal enzyme is GAA or comprises GAA activity, and the membrane protein is selected from the group consisting of CD63, APLP2, and PRLR.

12. The composition of any one of claims 1-11, wherein the lysosomal enzyme is GAA or comprises GAA activity, and the membrane protein is CD63.

13. The composition of any one of claims 1-12, wherein the lysosomal enzyme comprises the amino acid sequence of SEQ ID NO:1.

14. The composition of any one of claims 1-10, wherein the lysosomal enzyme is GLA or comprises GLA activity, and the membrane protein is selected from the group consisting of CD63, APLP2, and PRLR.

15. The composition of any one of claims 1-10 and 14, wherein the enzyme is GLA or comprises GLA activity, and the membrane protein is CD63.

16. The composition of any one of claims 1-10, 14 and 15, wherein the lysosomal enzyme comprises the amino acid sequence of SEQ ID NO:2.

17. A method of treating a subject suffering from a lysosomal storage disease (LSD) comprising administering to the subject a biotherapeutic complex comprising (a) an enzyme and (b) an antigen-binding protein that binds a membrane protein that undergoes endocytosis, wherein the biotherapeutic complex enters a lysosome of a cell of the subject and delivers to the lysosome the enzyme, which replaces the enzymatic activity that is associated with the LSD ("endogenous enzyme").

18. The method of claim 17, wherein the LSD is selected from the group consisting of a sphingolipidosis, a mucopolysaccharidosis, and a glycogen storage disease.

19. The method of claim 17 or 18, wherein the LSD is selected from the group consisting of Fabry disease, Gaucher disease type I, Gaucher disease type II, Gaucher disease type III, Niemann-Pick disease type A, Niemann-Pick disease type BGM1-gangliosidosis, Sandhoff disease, Tay-Sachs

disease, GM2-activator deficiency, GM3-gangliosidosis, metachromatic leukodystrophy, sphingolipid-activator deficiency, Scheie disease, Hurler-Sceie disease, Hurler disease, Hunter disease, Sanfilippo A, Sanfilippo B, Sanfilippo C, Sanfilippo D, Morquio syndrome A, Morquio syndrome B, Maroteaux-Lamy disease, Sly disease, MPS IX, and Pompe disease.

20. The method of any one of claims 17-19, wherein the LSD is Fabry disease or Pompe disease.

21. The method of claim 17, wherein the enzyme is selected from the group consisting of α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, saposin-C activator, ceramidase, sphingomyelinase, β-hexosaminidase, GM2 activator, GM3 synthase, arylsulfatase, sphingolipid activator, α-iduronidase, iduronidase-2-sulfatase, heparin *N*-sulfatase, *N*-acetyl-α-glucosaminidase, α-glucosamide *N*-acetyltransferase, *N*-acetylglucosamine-6- sulfatase, *N*-acetylgalactosamine-6-sulfate sulfatase, *N*-acetylgalactosamine-4-sulfatase, β-glucuronidase, and hyaluronidase.

22. The method of any one of claims 17-21, wherein the enzyme does not induce an immunological reaction in the subject.

23. The method of any one of claims 17-22, wherein the enzyme is an isozyme.

24. The method of claim 23, wherein the LSD is Pompe disease, the endogenous enzyme is α-glucosidase (GAA), and the isozyme is selected from the group consisting of acid α-glucosidase,

sucrase-isomaltase (SI), maltase-glucoamylase (MGAM), glucosidase II (GANAB), and neutral α-glucosidase (C GNAC).

25. The method of claim 23, wherein the LSD is Fabry disease, the endogenous enzyme is α-galactosidase A (GLA), and the isozyme is α-*N*-acetylgalactosaminidase engineered to gain GLA activity.

26. The method of any one of claims 17-25, wherein the membrane protein is selected from the group consisting of CD63, MHC-I, Kremen-1, Kremen-2, LRP5, LRP6, LRP8, transferrin receptor, LDL-receptor, LDL-related protein 1 receptor, ASGR1, ASGR2, amyloid precursor protein-like protein-2 (APLP2), apelin receptor (APLNR), PRLR (prolactin receptor), MAL (Myelin And Lymphocyte protein, a.k.a. VIP17), IGF2R, vacuolar-type H+ ATPase, diphtheria toxin receptor, folate receptor, glutamate receptors, glutathione receptor, leptin receptors, scavenger receptors, SCARA1-5, SCARB1-3, CD36, CDH16 (Cadheri-16), CLDN16 (Claudn-16), KL (Klotho), PTH1R (parathyroid hormone receptor), SLC22A13 (Solute carrier family 22 member 13), SLC5A2 (Sodium/glucose cotransporter 2), UMOD (Uromodulin), BMPR1A (Bone morphogenetic protein receptor 1A), m-cadherin, CD9, MuSK (muscle-specific kinase), LGR4/GPR48 (G protein-coupled receptor 48), cholinergic receptor (nicotinic) alpha 1, CDH15 (Cadheri-15), ITGA7 (Integrin alpha-7), CACNG1 (L-type calcium channel subunit gamma-1), CACNAls (L-type calcium channel subunit alpha-15), CACNG6 (L-type calcium channel subunit gamma-6), SCN1B (Sodium channel subunit beta-1), CHRNA1 (ACh receptor subunit alpha),

CHRND (ACh receptor subunit delta), LRRC14B (Leucine-rich repeat-containing protein 14B), and POPDC3 (Popeye domain-containing protein 3).

27. The method of any one of claims 17-26, wherein the membrane protein is CD63.

28. The method of any one of claims 17-26, wherein the membrane protein is APLP2.

29. The method of any one of claims 17-28, wherein the antigen-binding protein is an antibody, an antibody fragment, or other antigen-binding protein.

30. The method of claim 29, wherein the antigen-binding protein is a bispecific antibody that binds the enzyme and the membrane protein.

31. The method of any one of claims 17-29, wherein the enzyme is linked to an immunoglobulin Fc-domain and the antigen-binding protein comprises a half-antibody.

32. The method of any one of claims 17-29, wherein the enzyme is covalently linked to the C-terminus of the heavy chain of an anti-membrane protein antibody.

33. The method of any one of claims 17-29, wherein the enzyme is covalently linked to the N-terminus of the heavy chain of an anti-membrane protein antibody.

34. The method of claim 30, wherein the enzyme comprises GLA, the membrane protein is CD63, and the LSD is Fabry disease.

35. The method of claim 30, wherein the enzyme comprises GAA, the membrane protein is CD63, and the LSD is Pompe disease.