

## CLAIMS:

1. A therapeutic regimen useful for treatment of GM1 gangliosidosis in a human patient, wherein the regimen comprises administration of a recombinant adeno-associated virus (rAAV) vector having an AAV capsid and a vector genome comprising a sequence encoding human  $\beta$ -galactosidase under control of regulatory sequences that direct expression thereof in target cell, the administration comprising intra-cisterna magna (ICM) injection of a single dose comprising:
  - (i) about  $1.6 \times 10^{13}$  to about  $1.6 \times 10^{14}$  GC, wherein the patient is about 1 month to about 4 months of age;
  - (ii) about  $2.1 \times 10^{13}$  to about  $2.1 \times 10^{14}$  GC, wherein the patient is at least 4 months to under 8 months of age;
  - (iii) about  $2.6 \times 10^{13}$  to about  $2.6 \times 10^{14}$  GC, wherein the patient is at least 8 months up to 12 months of age; or
  - (iv) about  $3.2 \times 10^{13}$  to about  $3.2 \times 10^{14}$  GC, wherein the patient is at least 12 months of age.
2. The regimen according to claim 1, wherein the human  $\beta$ -galactosidase coding sequence comprises a nucleotide sequence set forth in SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 or a sequence at least 95% identical to any one of SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 that encodes the mature  $\beta$ -galactosidase of amino acids 24 to 677 of SEQ ID NO: 4.
3. The regimen according to claim 1 or 2, wherein the encoded human  $\beta$ -galactosidase has the sequence selected from:
  - (a) about amino acids 1 to 677 of SEQ ID NO: 4; and
  - (b) a synthetic human enzyme comprising a heterologous leader sequence fused to about amino acids 24 to 677 of SEQ ID NO: 4.
4. The regimen according to any one of claims 1 to 3, wherein the vector genome further comprises a 5' inverted terminal repeat (ITR) sequence, a regulatory element derived from the human ubiquitin C (UbC) promoter, a chimeric intron, a polyA signal, and/or a 3' ITR sequence.
5. The regimen according to any one of claims 1 to 4, wherein the patient has been identified as having type 1 (infantile) GM1 or type 2a (late infantile) GM1.

6. The regimen according to any one of claims 1 to 5, further comprising administration of at least one immunosuppressive co-therapy to the patient at least one day prior to or on the day of delivery of the rAAV.
7. The regimen according to claim 6, wherein the immunosuppressive co-therapy includes one or more corticosteroids.
8. The regimen according to claim 6 or 7, wherein the immunosuppressive co-therapy includes oral prednisolone.
9. The regimen according to claim 8, wherein the oral prednisolone is administered at about 1 mg/kg body weight.
10. The regimen according to any one of claims 5 to 9, wherein administration of the at least one immunosuppressive co-therapy continues for at least 3 to 4 weeks following administration of the rAAV.
11. The regimen according to any one of claims 1 to 10, wherein the efficacy of treatment is assessed by one or more of a delay in the onset of seizures, a decrease in the frequency of seizures,  $\beta$ -galactosidase in serum and/or cerebral spinal fluid, and volumetric changes in brain tissue as measured by magnetic resonance imaging (MRI).
12. A composition comprising a recombinant AAV(rAAV) vector comprising an AAV capsid and a vector genome comprising a human  $\beta$ -galactosidase coding sequence and expression control sequences that direct expression thereof in target cells, wherein the rAAV vector is formulated for intra-cisterna magna (ICM) injection to a human subject in need thereof to administer a dose of:
  - (i) about  $1.6 \times 10^{13}$  to about  $1.6 \times 10^{14}$  GC, wherein the patient is about 1 month to about 4 months of age;
  - (ii) about  $2.1 \times 10^{13}$  to about  $2.1 \times 10^{14}$  GC, wherein the patient is at least 4 months to under 8 months of age;
  - (iii) about  $2.6 \times 10^{13}$  to about  $2.6 \times 10^{14}$  GC, wherein the patient is at least 8 months up to 12 months of age; or
  - (iv) about  $3.2 \times 10^{13}$  to about  $3.2 \times 10^{14}$  GC, wherein the patient is at least 12 months of age.

13. The composition according to claim 12, wherein the human  $\beta$ -galactosidase coding sequence comprises a nucleotide sequence set forth in SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 or a sequence at least 95% identical to any one of SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 that encodes the mature  $\beta$ -galactosidase of amino acids 24 to 677 of SEQ ID NO: 4.

14. The composition according to claim 12 or 13, wherein the vector genome further comprises a 5' inverted terminal repeat (ITR) sequence, a regulatory element derived from the human ubiquitin C (UbC) promoter, a chimeric intron, a polyA signal, and/or a 3' ITR sequence.

15. The composition according to any one of claims 12 to 14, wherein the rAAV is formulated in a suspension to deliver  $3.33 \times 10^{10}$  GC per gram of brain mass to  $3.33 \times 10^{11}$  GC per gram of brain mass, optionally wherein the volume of the administered dose is about 3.0 mL to about 5.0 mL.

16. The composition according to any one of claims 12 to 15, wherein the rAAV is in a formulation buffer having a pH of 6 to 9, optionally wherein the pH is about 7.2.

17. The composition according to any one of claims 12 to 16 for use in a co-therapy comprising administration of at least one immunosuppressant to the patient at least one day prior to or on the day of delivery of the rAAV.

18. The composition according to claim 17, wherein the immunosuppressant is a corticosteroid, optionally orally delivered prednisolone.

19. A method of treating a patient with GM1 gangliosidosis, the method comprising administering a single dose of a recombinant adeno-associated virus (rAAV) to the patient by intracisternal magna (ICM) injection,

wherein the rAAV comprises an AAV capsid and a vector genome comprising a sequence encoding human  $\beta$ -galactosidase under control of regulatory sequences that direct expression thereof in a target cell, and

wherein the single dose is from  $1 \times 10^{10}$  GC to  $3.4 \times 10^{11}$  GC per gram of estimated brain mass of the patient.

20. The method of claim 19, wherein the patient has onset of a GM1 symptom at or before 18 months of age.

21. The method of claim 20, wherein the patient has onset of a GM1 symptom at 6 months of age or earlier.
22. The method of claim 20, wherein the patient has onset of a GM1 symptom at 6 to 18 months of age.
23. The method of any one of claims 19 to 21, wherein the patient has type1 (infantile) GM1.
24. The method of any one of claims 19, 20, or 22, wherein the patient has type2a (late infantile) GM1.
25. The method of any one of claims 19 to 24, wherein the patient has been diagnosed to have type 1 or type 2a GM1.
26. The method of any one of claims 19 to 25, wherein the subject is at least 4 months of age.
27. The method of claim 26, wherein the subject is 4 to 36 months of age.
28. The method of claim 26, wherein the subject is a human patient of 4 to 24 months of age.
29. The method of claim 26, wherein the patient is a human patient of 6 to 36 months of age.
30. The method of claim 26, wherein the patient is a human patient of 6 to 24 months of age.
31. The method of claim 26, wherein the patient is a human patient of 12 to 36 months of age.
32. The method of claim 26, wherein the patient is a human patient of 12 to 24 months of age.
33. The method of any one of claims 19 to 32, wherein the single dose is  $3.3 \times 10^{10}$  GC per gram of estimated brain mass of the patient.
34. The method of claim 33, wherein the single dose is  $2.1 \times 10^{13}$  to  $2.5 \times 10^{13}$  GC of the rAAV.
35. The method of claim 33, wherein the single dose is  $2.6 \times 10^{13}$  to  $3.1 \times 10^{13}$  GC of the rAAV.

36. The method of claim 33, wherein the single dose is  $3.2 \times 10^{13}$  to  $4.5 \times 10^{13}$  GC of the rAAV.
37. The method of any one of claims 19 to 32, wherein the single dose is  $1.11 \times 10^{11}$  GC per gram of estimated brain mass of the patient.
38. The method of claim 37, wherein the single dose is  $6.8 \times 10^{13}$  to  $8.6 \times 10^{13}$  GC of the rAAV.
39. The method of claim 37, wherein the single dose is  $8.7 \times 10^{13}$  to  $0.9 \times 10^{14}$  GC of the rAAV.
40. The method of claim 37, wherein the single dose is  $1.0 \times 10^{14}$  to  $1.5 \times 10^{14}$  GC of the rAAV.
41. The method of any one of claims 19 to 25, wherein the patient is 4 to 8 months of age, and the single dose is  $2.1 \times 10^{13}$  GC of the rAAV.
42. The method of any one of claims 19 to 25, wherein the patient is 4 to 8 months of age, and the single dose is  $6.8 \times 10^{13}$  GC of the rAAV.
43. The method of any one of claims 19 to 25, wherein the patient is 8 to 12 months of age, and the single dose is  $2.6 \times 10^{13}$  GC of the rAAV.
44. The method of any one of claims 19 to 25, wherein the patient is 8 to 12 months of age, and the single dose is  $8.7 \times 10^{13}$  GC of the rAAV.
45. The method of any one of claims 19 to 25, wherein the patient is at least 12 months of age, and the single dose is  $3.2 \times 10^{13}$  GC of the rAAV.
46. The method of any one of claims 19 to 25, wherein the patient is at least 12 months of age, and the single dose is  $1.0 \times 10^{14}$  GC of the rAAV.
47. The method of any one of claims 19 to 46, further comprising the step of hematopoietic stem cell transplantation.
48. The method of any one of claims 19 to 47, further comprising the step of administering a steroid to the patient.
49. The method of claim 48, wherein the steroid is a corticosteroid.
50. The method of claim 48 or 49, wherein the steroid is administered systematically daily for at least 21 days.

51. The method of claim 48 or 49, wherein the steroid is administered systematically daily for 30 days.
52. The method of any one of claims 19 to 51, wherein the sequence encoding human  $\beta$ -galactosidase comprises a nucleotide sequence set forth in SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 or a sequence at least 95% identical to any one of SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 that encodes the mature  $\beta$ -galactosidase of amino acids 24 to 677 of SEQ ID NO: 4.
53. The method of any one of claims 19 to 51, wherein the human  $\beta$ -galactosidase has an amino acid sequence of SEQ ID NO: 4 or a functional fragment thereof.
54. The method of any one of claims 19 to 51, wherein the vector genome has a sequence selected from SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14 or SEQ ID NO: 15.
55. The method of any one of claims 19 to 51, wherein the vector genome has a sequence at least 95% identical to SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15.
56. The method of any one of claims 19 to 51, wherein the vector genome further comprises a 5' inverted terminal repeat (ITR) sequence, a regulatory element derived from the human ubiquitin C (UbC) promoter, a chimeric intron, a polyA signal, and/or a 3' ITR sequence.
57. A pharmaceutical composition in a unit dosage form, comprising:  $1 \times 10^{13}$  GC to  $5 \times 10^{14}$  of a recombinant adeno-associated virus (rAAV) vector in a buffer, wherein the rAAV comprises an AAV capsid and a vector genome comprising a sequence encoding human  $\beta$ -galactosidase under control of regulatory sequences that direct expression thereof in a target cell.
58. The pharmaceutical composition of claim 57, formulated for intracisternal magna (ICM) injection.
59. The pharmaceutical composition of claim 58, wherein the buffer comprises sodium phosphate, sodium chloride, potassium chloride, calcium chloride, magnesium chloride, and poloxamer 188.
60. The pharmaceutical composition of any one of claims 57 to 59, wherein the buffer comprises 1 mM sodium phosphate, 150 mM sodium chloride, 3 mM potassium

chloride, 1.4 mM calcium chloride, 0.8 mM magnesium chloride, and 0.001% poloxamer 188.

61. The pharmaceutical composition of any one of claims 57 to 60, in a liquid form.

62. The pharmaceutical composition of claim 61, having 3.0 mL, 4.0mL or 5.0 mL of volume.

63. The pharmaceutical composition of claim 57 to 62, comprising  $2.1 \times 10^{13}$  to  $2.5 \times 10^{13}$  GC of the rAAV.

64. The pharmaceutical composition of claim 57 to 62, comprising  $2.6 \times 10^{13}$  to  $3.1 \times 10^{13}$  GC of the rAAV.

65. The pharmaceutical composition of claim 57 to 62, comprising  $3.2 \times 10^{13}$  to  $4.5 \times 10^{13}$  GC of the rAAV.

66. The pharmaceutical composition of claim 57 to 62, comprising  $6.8 \times 10^{13}$  to  $8.6 \times 10^{13}$  GC of the rAAV.

67. The pharmaceutical composition of claim 57 to 62, comprising  $8.7 \times 10^{13}$  to  $0.9 \times 10^{14}$  GC of the rAAV.

68. The pharmaceutical composition of claim 57 to 62, comprising  $1.0 \times 10^{14}$  to  $1.5 \times 10^{14}$  GC of the rAAV.

69. The pharmaceutical composition of any one of claims 57 to 68, wherein the sequence encoding human  $\beta$ -galactosidase comprises a nucleotide sequence set forth in SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 or a sequence at least 95% identical to any one of SEQ ID NO: 8, SEQ ID NO: 7, SEQ ID NO: 6, or SEQ ID NO: 5 that encodes the mature  $\beta$ -galactosidase of amino acids 24 to 677 of SEQ ID NO: 4.

70. The pharmaceutical composition of any one of claims 57 to 68, wherein the human  $\beta$ -galactosidase has an amino acid sequence of SEQ ID NO: 4 or a functional fragment thereof.

71. The pharmaceutical composition of any one of claims 57 to 68, wherein the vector genome has a sequence selected from SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15.

72. The pharmaceutical composition of any one of claims 57 to 68, wherein the vector genome has a sequence at least 95% identical to SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, or SEQ ID NO: 15.

73. The pharmaceutical composition of any one of claims 57 to 68, wherein the vector genome further comprises a 5' inverted terminal repeat (ITR) sequence, a regulatory element derived from the human ubiquitin C (UbC) promoter, a chimeric intron, a polyA signal, and/or a 3' ITR sequence.