response against the mini-dystrophin protein was stimulated in three (Bowles, et al., Mol Ther. 20(2):443-455 (2012)).

[00011] Thus, there exists a need in the art for AAV vectors encoding mini-dystrophins that can be expressed at high levels in transduced cells of subjects with DMD while minimizing immune responses to the mini-dystrophin protein.

Summary

[00011a] In one aspect, the present disclosure refers to a polynucleotide comprising a nucleic acid sequence encoding a human mini-dystrophin protein, wherein said mini-dystrophin protein comprises the following domains from full-length wild type human muscle dystrophin protein in order from amino-terminus to carboxy-terminus: N-terminal Actin-Binding Domain, hinge H1, rod R1, rod R2, hinge H3, rod R22, rod R23, rod R24, hinge H4, Cysteine-Rich Domain, and the Carboxy-Terminal Domain not including the last 3 amino acids in wild type human muscle dystrophin

[00011b] In another aspect, the present disclosure refers to an AAV vector genome comprising the polynucleotide as disclosed herein and at least one AAV inverted terminal repeat (ITR).

[00011c] In yet another aspect, the present disclosure refers to a recombinant AAV particle comprising an AAV capsid and the vector genome as disclosed herein.

[00011d] In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising the recombinant AAV particle as disclosed herein and a pharmaceutically acceptable carrier.

[00011e] In yet another aspect, the present disclosure refers to a recombinant AAV particle comprising an AAV9 capsid and a single-stranded DNA vector genome comprising a first ITR from AAV2, a genetic control region conferring tissue-specific expression in muscle and heart operably linked with a human codon-optimized nucleic acid sequence encoding a human mini-dystrophin protein consisting of the amino acid sequence of SEQ ID NO:7, a polyadenylation signal transcription termination sequence, and a second ITR from AAV2.

[00011f] In yet another aspect, the present disclosure refers to a method of making a recombinant AAV particle comprising an AAV9 capsid and a vector genome, comprising: introducing into a cell a polynucleotide comprising the AAV vector genome

as disclosed herein, a polynucleotide comprising an AAV rep gene, and a polynucleotide comprising an AAV9 cap gene, incubating said cell; and purifying the recombinant AAV particles produced thereby.

[00011g] In yet another aspect, the present disclosure refers to a recombinant AAV particle produced by the method as disclosed herein.

[00011h] In yet another aspect, the present disclosure refers to a recombinant AAV particle, comprising an AAV9 capsid and a vector genome comprising the nucleotide sequence of SEQ ID NO:18 or the reverse complement thereof.

[00011i] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD).

[00011j] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the average of at least one of ALT, AST, or LDH levels in the blood of a subject to within an average selected from the group consisting of 7-, 6-, 5-, 4-, 3-, and 2-fold greater than the average level of ALT, AST, or LDH in the blood of healthy controls.

[00011k] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the total CK level in the blood of a subject to within a level selected from the group consisting of 50-, 48-, 46-, 44-, 42-, 40-, 38-, 36-, 34-, 32-, 30-, 28-, 26-, 24-, 22-, 20-, 18-, 16-, 14-, 12-, 10-, 9-, 8-, 7-, 6-, 5-, 4-, 3-, and 2-fold greater than the average total CK level in the blood of healthy controls.

[00011l] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to increase the average 6 minute walk distance (6MWD) of a subject by at least a distance selected from the group consisting of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, and 100 meters compared to the average 6MWD of untreated DMD controls, or of the subject before treatment.

[00011m] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the average time required for a subject to perform the 4 stair climb test by at least a time selected from the group consisting of 0,2, 0,4, 0,6, 0,8, 1,0, 1,2, 1,4, 1,6, 1,8, 2,0, 2,2, 2,4, 2,6, 2,8, 3,0, 3,2, 3,4, 3,6, 3,8, and 4,0 seconds compared to the average time of untreated DMD controls, or the subject before treatment.

[00011n] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the average proportion of treated subjects that have lost ambulation by at least a reduction selected from the group consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% and 65% compared to the average proportion of untreated DMD controls that have lost ambulation.

[00011o] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the fat fraction in the lower extremities of a subject by at least a reduction selected from the group consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% and 75% compared to the average fat fraction in the lower extremities of untreated DMD controls, or the subject before treatment.

[00011p] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to cause at least a percentage selected from the group consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% and 90% of the muscle fibers in a skeletal muscle biopsy from a subject to produce detectable levels of said mini-dystrophin protein.

[00011q] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use causes a cellular immune response against said mini-dystrophin protein, or muscle inflammation, in less than or

equal to a percentage selected from the group consisting of 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% and 20% of treated subjects.

[00011r] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for increasing muscle mass or strength in a subject.

[00011s] In yet another aspect, the present disclosure refers to use of the pharmaceutical composition as disclosed herein in the manufacture of a medicament for preventing loss of muscle mass or strength in a subject.

[00012] Disclosed and exemplified herein are mini-dystrophin proteins, codon-optimized genes for expressing such mini-dystrophin proteins, AAV vectors for transducing cells with such genes, and methods of prevention and treatment using such AAV vectors, in particular for preventing and treating dystrophinopathies in subjects in need thereof. In some of these examples, AAV vectors of the disclosure are capable of guiding production of significant levels of mini-dystrophin in transduced cells while causing no or only muted immune response against the mini-dystrophin protein.

[00013] Certain non-limiting examples (E) of the disclosure are set forth below. These and related examples are described in further detail in the Detailed Description, including the Examples and Drawings.

E1. A mini-dystrophin protein comprising, consisting essentially of, or consisting of the N-terminus, the Actin Binding Domain (ABD), hinge H1, rods R1 and R2, hinge H3, rods R22, R23, and R24, hinge H4, the cysteine-rich (CR) domain, and a portion of the carboxy-terminal (CT) domain of wildtype human muscle dystrophin protein (SEQ ID NO:25), wherein the CT domain does not comprise the last three amino acid residues at the carboxy-terminus of wildtype dystrophin protein.

E2. The mini-dystrophin protein of E1, wherein the N-terminus and Actin Binding Domain (ABD) together comprise, consist essentially of, or consist of amino acid numbers 1-240 from SEQ ID NO:25; hinge H1 comprises, consists essentially of, or consists of amino acid numbers 253-327 from SEQ ID NO:25; rod R1 comprises, consists essentially of, or consists of amino acid numbers 337-447 from SEQ ID NO:25; rod R2 comprises, consists essentially of, or consists of amino acid numbers 448-556 from SEQ ID NO:25; hinge H3 comprises, consists essentially of, or consists of amino acid numbers 2424-2470 from SEQ ID NO:25; rod R22 comprises, consists essentially of, or consists of amino acid numbers 2687-2802 from SEQ ID NO:25; rod R23 comprises, consists essentially of, or consists of amino acid numbers 2803-2931 from SEQ ID NO:25; rod

E62. The rAAV particle of E61, wherein the transcription termination sequence comprises a polyadenylation signal.

E63. The rAAV particle of any one of E44-E62, wherein the vector genome comprises in 5′ to 3′ order: a first AAV2 ITR, a muscle-specific transcriptional regulatory element operably linked to a human codon-optimized nucleic acid sequence encoding a human mini-dystrophin protein, a transcription termination sequence, and a second AAV2 ITR.

E64. The rAAV particle of E63, wherein the muscle-specific transcriptional regulatory element comprises the nucleic acid sequence of SEQ ID NO:16.

E65. The rAAV particle of examples E63 or E64, wherein the human codon-optimized nucleic acid sequence comprises the nucleic acid sequence of SEQ ID NO:1.

E66. The rAAV particle of examples E63-E65, wherein the transcription termination sequence comprises the nucleic acid sequence of SEQ ID NO:17.

E67. The rAAV particle of any one of E44-E48, E51, and E53-E66, wherein the vector genome comprises the nucleic acid sequence of SEQ ID NO:18 or the reverse-complement thereof.

E68. The rAAV particle of any one of E44-E48, E51, and E53-E66, wherein the vector genome consists essentially of the nucleic acid sequence of SEQ ID NO:18 or the reverse-complement thereof.

E69. The rAAV particle of any one of E44-E48, E51, and E53-E66, wherein the vector genome consists of the nucleic acid sequence of SEQ ID NO:18 or the reverse-complement thereof.

E70. A recombinant AAV particle, comprising an AAV9 capsid and a vector genome comprising the nucleic acid sequence of SEQ ID NO:18 or the reverse complement thereof.

E71. A recombinant AAV particle, comprising an AAV9 capsid and a vector genome consisting essentially of the nucleic acid sequence of SEQ ID NO:18 or the reverse complement thereof.

E72. A recombinant AAV particle, comprising an AAV9 capsid and a vector genome consisting of the nucleic acid sequence of SEQ ID NO:18 or the reverse complement thereof.

E73. A pharmaceutical composition comprising the rAAV particle of any one of E42-E72 and a pharmaceutically acceptable carrier.

THAT WHICH IS CLAIMED IS:

1. A polynucleotide comprising a nucleic acid sequence encoding a human mini-dystrophin protein, wherein said mini-dystrophin protein comprises the following domains from full-length wild type human muscle dystrophin protein in order from amino-terminus to carboxy-terminus: N-terminal Actin-Binding Domain, hinge H1, rod R1, rod R2, hinge H3, rod R22, rod R23, rod R24, hinge H4, Cysteine-Rich Domain, and the Carboxy-Terminal Domain not including the last 3 amino acids in wild type human muscle dystrophin.

2. The polynucleotide of claim 1, wherein the N-terminal Actin Binding Domain of said mini-dystrophin protein comprises amino acid numbers 1-240 from SEQ ID NO:25.

3. The polynucleotide of claim 1, wherein the H1 domain of said mini-dystrophin protein comprises amino acid numbers 253-327 from SEQ ID NO:25.

4. The polynucleotide of claim 1, wherein the R1 domain of said mini-dystrophin protein comprises amino acid numbers 337-447 from SEQ ID NO:25.

5. The polynucleotide of claim 1, wherein the R2 domain of said mini-dystrophin protein comprises amino acid numbers 448-556 from SEQ ID NO:25.

6. The polynucleotide of claim 1, wherein the H3 domain of said mini-dystrophin protein comprises amino acid numbers 2424-2470 from SEQ ID NO:25.

7. The polynucleotide of claim 1, wherein the R22 domain of said mini-dystrophin protein comprises amino acid numbers 2687-2802 from SEQ ID NO:25.

8. The polynucleotide of claim 1, wherein the R23 domain of said mini-dystrophin protein comprises amino acid numbers 2803-2931 from SEQ ID NO:25.

9. The polynucleotide of claim 1, wherein the R24 domain of said mini-dystrophin protein comprises amino acid numbers 2932-3040 from SEQ ID NO:25.,

10. The polynucleotide of claim 1, wherein the H4 domain of said mini-dystrophin protein comprises amino acid numbers 3041-3112 from SEQ ID NO:25.

11. The polynucleotide of claim 1, wherein the Cysteine-Rich domain of said mini-dystrophin protein comprises amino acid numbers 3113-3299 from SEQ ID NO:25.

12. The polynucleotide of claim 1, wherein the Carboxy-Terminal domain of said mini-dystrophin protein comprises amino acid numbers 3300-3408 from SEQ ID NO:25.

13. The polynucleotide of claim 2, wherein the H1 domain of said mini-dystrophin protein comprises amino acid numbers 253-327 from SEQ ID NO:25.

14. The polynucleotide of claim 13, wherein the R1 domain of said mini-dystrophin protein comprises amino acid numbers 337-447 from SEQ ID NO:25.

15. The polynucleotide of claim 14, wherein the R2 domain of said mini-dystrophin protein comprises amino acid numbers 448-556 from SEQ ID NO:25.

16. The polynucleotide of claim 15, wherein the H3 domain of said mini-dystrophin protein comprises amino acid numbers 2424-2470 from SEQ ID NO:25.

17. The polynucleotide of claim 16, wherein the R22 domain of said mini-dystrophin protein comprises amino acid numbers 2687-2802 from SEQ ID NO:25.

18. The polynucleotide of claim 17, wherein the R23 domain of said mini-dystrophin protein comprises amino acid numbers 2803-2931 from SEQ ID NO:25.

19. The polynucleotide of claim 18, wherein the R24 domain of said mini-dystrophin protein comprises amino acid numbers 2932-3040 from SEQ ID NO:25.

20. The polynucleotide of claim 19, wherein the H4 domain of said mini-dystrophin protein comprises amino acid numbers 3041-3112 from SEQ ID NO:25.

21. The polynucleotide of claim 20, wherein the Cysteine-Rich domain of said mini-dystrophin protein comprises amino acid numbers 3113-3299 from SEQ ID NO:25.

22. The polynucleotide of claim 21, wherein the Carboxy-Terminal domain of said mini-dystrophin protein comprises amino acid numbers 3300-3408 from SEQ ID NO:25.

23. The polynucleotide of claim 22, wherein the human mini-dystrophin protein comprises the amino acid sequence of SEQ ID NO:7.

24. The polynucleotide of claim 22, wherein the human mini-dystrophin protein consists of the amino acid sequence of SEQ ID NO:7.

25. The polynucleotide of claim 23, further comprising a muscle-specific transcriptional regulatory element operably linked to said nucleic acid sequence encoding the human mini-dystrophin protein.

26. The polynucleotide of claim 25, wherein said muscle-specific transcriptional regulatory element is derived from the human or mouse creatine kinase gene.

27. The polynucleotide of claim 26, wherein said muscle-specific transcriptional regulatory element comprises the nucleotide sequence of SEQ ID NO:16.

28. The polynucleotide of claim 25, further comprising a transcription termination sequence.

29. The polynucleotide of claim 28, wherein said transcription termination sequence comprises the nucleotide sequence of SEQ ID NO:17.

30. The polynucleotide of claim 23, further comprising an intron.

31. The polynucleotide of claim 23, wherein said nucleic acid sequence encoding said human mini-dystrophin is codon-optimized.

32. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 90% identical to the nucleic acid sequence of SEQ ID NO:1.

33. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 91% identical to the nucleic acid sequence of SEQ ID NO:1.

34. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 92% identical to the nucleic acid sequence of SEQ ID NO:1.

35. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 93% identical to the nucleic acid sequence of SEQ ID NO:1.

36. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 94% identical to the nucleic acid sequence of SEQ ID NO:1.

37. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 95% identical to the nucleic acid sequence of SEQ ID NO:1.

38. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 96% identical to the nucleic acid sequence of SEQ ID NO:1.

39. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 97% identical to the nucleic acid sequence of SEQ ID NO:1.

40. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 98% identical to the nucleic acid sequence of SEQ ID NO:1.

41. The polynucleotide of claim 31, wherein said nucleic acid sequence is at least 99% identical to the nucleic acid sequence of SEQ ID NO:1.

42. An AAV vector genome comprising the polynucleotide of claim 28 and at least one AAV inverted terminal repeat (ITR).

43. The AAV vector genome of claim 42 further comprising a second AAV ITR.

44. The AAV vector genome of claim 43, wherein each of said ITRs are from AAV2.

45. A recombinant AAV particle comprising an AAV capsid and the vector genome of claim 42.

46. The recombinant AAV particle of claim 45, wherein said capsid is from AAV9.

47. A pharmaceutical composition comprising the recombinant AAV particle of claim 46 and a pharmaceutically acceptable carrier.

48. A recombinant AAV particle comprising an AAV9 capsid and a single-stranded DNA vector genome comprising a first ITR from AAV2, a genetic control region conferring tissue-specific expression in muscle and heart operably linked with a human codon-optimized nucleic acid sequence encoding a human mini-dystrophin protein consisting of the amino acid sequence of SEQ ID NO:7, a polyadenylation signal transcription termination sequence, and a second ITR from AAV2.

49. The recombinant AAV particle of claim 48, wherein the nucleic acid sequence encoding the human mini-dystrophin protein is at least 90% identical to the nucleic acid sequence of SEQ ID NO:1.

50. The recombinant AAV particle of claim 48, wherein the polyadenylation signal sequence comprises the nucleic acid sequence of SEQ ID NO:17.

51. The recombinant AAV particle of claim 48, wherein the nucleic acid sequence encoding the human mini-dystrophin protein is at least 95% identical to the nucleic acid sequence of SEQ ID NO:1.

52. The recombinant AAV particle of claim 48, wherein the genetic control region is a synthetic enhancer and promoter derived from a muscle creatinine kinase gene.

53. The recombinant AAV particle of claim 52, wherein the synthetic enhancer and promoter comprises the nucleic acid sequence of SEQ ID NO:16.

54. A method of making a recombinant AAV particle comprising an AAV9 capsid and a vector genome, comprising:
introducing into a cell a polynucleotide comprising the AAV vector genome of claim 42, a polynucleotide comprising an AAV rep gene, and a polynucleotide comprising an AAV9 cap gene,
incubating said cell; and
purifying the recombinant AAV particles produced thereby.

55. The method of claim 54, further comprising introducing into the cell a polynucleotide encoding one or more viral helper genes.

56. A recombinant AAV particle produced by the method of claim 54 or 55.

57. A recombinant AAV particle, comprising an AAV9 capsid and a vector genome comprising the nucleotide sequence of SEQ ID NO:18 or the reverse complement thereof.

58. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD).

59. The use of claim 58, wherein the composition is to be administered with a least a second agent effective for treating DMD.

60. The use of claim 59, wherein said second agent is selected from the group consisting of: an antisense oligonucleotide that causes exon skipping of the *DMD* gene, an anti-myostatin antibody, an agent that promotes ribosomal read-through of nonsense mutations, an agent that suppresses premature stop codons, an anabolic steroid and a corticosteroid.

61. The use of claim 58, wherein the recombinant AAV particles are to be administered in a dose selected from the group consisting of: $1x10^{12}$ vg/kg, $2x10^{12}$ vg/kg, $3x10^{12}$ vg/kg, $4x10^{12}$ vg/kg, $5x10^{12}$ vg/kg, $6x10^{12}$ vg/kg, $7x10^{12}$ vg/kg, $8x10^{12}$ vg/kg, $9x10^{12}$ vg/kg, $1x10^{13}$ vg/kg, $2x10^{13}$ vg/kg, $3x10^{13}$ vg/kg, $4x10^{13}$ vg/kg, $5x10^{13}$ vg/kg, $6x10^{13}$ vg/kg, $7x10^{13}$ vg/kg, $8x10^{13}$ vg/kg, $9x10^{13}$ vg/kg, $1x10^{14}$ vg/kg, $1,5x10^{14}$ vg/kg, $2x10^{14}$ vg/kg, $2,5x10^{14}$ vg/kg, $3x10^{14}$ vg/kg, $3,5x10^{14}$ vg/kg, $4x10^{14}$ vg/kg, $4,5x10^{14}$ vg/kg, $5x10^{14}$ vg/kg, $5,5x10^{14}$ vg/kg, $6x10^{14}$ vg/kg, $6,5x10^{14}$ vg/kg, $7x10^{14}$ vg/kg, $7,5x10^{14}$ vg/kg, $8x10^{14}$ vg/kg, $8,5x10^{14}$ vg/kg, and $9x10^{14}$ vg/kg.

62. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the average of at least one of ALT, AST, or LDH levels in the blood of a subject to within an average selected from the group consisting of 7-, 6-, 5-, 4-, 3-, and 2-fold greater than the average level of ALT, AST, or LDH in the blood of healthy controls.

63. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the total CK level in the blood of a subject to within a level selected from the group consisting of 50-, 48-, 46-, 44-, 42-, 40-, 38-, 36-, 34-, 32-, 30-, 28-, 26-, 24-, 22-, 20-, 18-, 16-, 14-, 12-, 10-, 9-, 8-, 7-, 6-, 5-, 4-, 3-, and 2-fold greater than the average total CK level in the blood of healthy controls.

64. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to increase the average 6 minute walk distance (6MWD) of a subject by at least a distance selected from the group consisting of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, and 100 meters compared to the average 6MWD of untreated DMD controls, or of the subject before treatment.

65. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the average time required for a subject to perform the 4 stair climb test by at least a time selected from the group consisting of 0,2, 0,4, 0,6, 0,8, 1,0, 1,2, 1,4, 1,6, 1,8, 2,0, 2,2, 2,4, 2,6, 2,8, 3,0, 3,2, 3,4, 3,6, 3,8, and 4,0 seconds compared to the average time of untreated DMD controls, or the subject before treatment.

66. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the average proportion of treated subjects that have lost ambulation by at least a reduction selected from the group consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60% and 65% compared to the average proportion of untreated DMD controls that have lost ambulation.

67. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to reduce the fat fraction in the lower extremities of a subject by at least a reduction selected from the group consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70% and 75% compared to the average fat fraction in the lower extremities of untreated DMD controls, or the subject before treatment.

68. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use is effective to cause at least a percentage selected from the group consisting of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%,

45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% and 90% of the muscle fibers in a skeletal muscle biopsy from a subject to produce detectable levels of said mini-dystrophin protein.

69. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for treating Duchenne muscular dystrophy (DMD), wherein said use causes a cellular immune response against said mini-dystrophin protein, or muscle inflammation, in less than or equal to a percentage selected from the group consisting of 0%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% and 20% of treated subjects.

70. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for increasing muscle mass or strength in a subject.

71. Use of the pharmaceutical composition of claim 47 in the manufacture of a medicament for preventing loss of muscle mass or strength in a subject.