CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition, comprising a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO) having a sequence according to SEQ ID NO: 1 (5'-GTXGCCCCTTCTCCCXGCAGC-3') in which all internucleoside linkages are *O,O*-linked phosphorothioate linkages and X is 5-methyl 2'-deoxycytidine, wherein the plurality of diastereomers has a phosphorus-31 nuclear magnetic resonance (^{31}P-NMR) spectrum comprising:
   a) one or more resonances between 54.8 to 55.5 ppm; and
   b) a first principal component 1 (PC1) score and a second principal component 2 (PC2) score, wherein:
      (i) the PC1 score is below about -0.20 or above about 0.25 and the PC2 score is below about 0.00; or
      (ii) the PC1 score is outside of the range of -0.32 to 0.30 and/or the PC2 score that is outside the range of 0.00 to 0.20.

2. The pharmaceutical composition of claim 1, wherein the ^{31}P-NMR spectrum comprises a PC1 score that is below about -0.20 and a PC2 score that is below about 0.00.

3. The pharmaceutical composition of claim 1 or 2, wherein the ^{31}P-NMR spectrum comprises a PC1 score that is from about -0.47 to about -0.20 and a PC2 score that is from about -0.27 to about 0.00.

4. The pharmaceutical composition of claim 1, wherein the ^{31}P-NMR spectrum comprises a PC1 score that is above about 0.25 and a PC2 score that is below about 0.00.

5. The pharmaceutical composition of claim 1 or 4, wherein the ^{31}P-NMR spectrum comprises a PC1 score that is from about 0.65 to about 0.9 and a PC2 score that is from about -0.47 to about 0.00.

6. The pharmaceutical composition of claim 1, wherein the ^{31}P-NMR spectrum comprises a PC1 score that is outside of the range of -0.32 to 0.30 when the PC2 score is above 0.00 and below 0.20.

7. The pharmaceutical composition of claim 1 or 6, wherein the ^{31}P-NMR spectrum comprises a PC2 score is outside the range of 0.00 to 0.20 when the PC1 is above -0.32 and below 0.30.

8. The pharmaceutical composition of any one of claims 1-7, wherein the ^{31}P-NMR spectrum comprises two or more resonances between 54.8 to 55.5 ppm.

9. The pharmaceutical composition of claim 8, wherein the two or more resonances between 54.8 to 55.5 ppm have different intensities.

10. The pharmaceutical composition of any one of claims 1-9, wherein the ^{31}P-NMR spectrum comprises, independently at about 54.8 ppm, at about 54.9 ppm, at about 55.0 ppm, at about 55.1 ppm, at about 55.2 ppm, at about 55.3 ppm, at about 55.4 ppm, and/or at about 55.5 ppm, one or more resonances.

11. The pharmaceutical composition of any one of claims 1-10, wherein the ^{31}P-NMR spectrum comprises, independently at about 54.8 ppm, at about 54.9 ppm, at about 55.0 ppm, at about 55.1 ppm, at about 55.2 ppm, at about 55.3 ppm, at about 55.4 ppm, and/or at about 55.5 ppm, two or more resonances.

12. The pharmaceutical composition of any one of claims 1-11, comprising 100 millimoles to 5 moles of SMAD7 ASO.

13. The pharmaceutical composition of any one of claims 1-12, comprising 100 millimoles to 2 moles, 100 millimoles to 900 millimoles, 300 millimoles to 5 moles, 300 millimoles to 2 moles, 300 millimoles to 900 millimoles, 900 millimoles to 5 moles, or 900 millimoles to 2 moles of SMAD7 ASO.

14. The pharmaceutical composition of any one of claims 1-13, comprising about 300 millimoles of SMAD7 ASO.

15. The pharmaceutical composition of any one of claims 1-13, comprising about 900 millimoles of SMAD7 ASO.

16. The pharmaceutical composition of any one of claims 1-13, comprising about 2 moles of SMAD7 ASO.

17. The pharmaceutical composition of any one of claims 1-13, comprising greater than 300 millimoles, greater than 900 millimoles, or greater than 2 moles of SMAD7 ASO.

18. The pharmaceutical composition of any one of claims 1-17, comprising:
    a) about 0.5% to about 30% by weight of the SMAD7 ASO;
    b) about 20% to about 50% by weight mannitol;

c) about 10% to about 30% by weight microcrystalline cellulose; and

d) an enteric coating comprising an ethylacrylate-methacrylic acid copolymer.

19. The pharmaceutical composition of any one of claims 1-18, comprising:

a) an intra-granular phase comprising:

i) about 5% to about 10% by weight of the SMAD7 ASO;

ii) about 40% by weight mannitol;

iii) about 8% by weight microcrystalline cellulose;

iv) about 5% by weight hydroxypropyl methylcellulose; and

v) about 2% by weight sodium starch glycolate;

b) an extra-granular phase comprising:

i) about 17% by weight microcrystalline cellulose;

ii) about 2% by weight sodium starch glycolate,

iii) about 0.4% by weight magnesium stearate; and

c) an enteric coating comprising an ethylacrylate-methacrylic acid copolymer,

wherein the percentages by weight are the weights of the ingredients compared to the total weight of the pharmaceutical composition.

20. The pharmaceutical composition of any one of claims 1-18, comprising:

a) about 5% to about 30% by weight of the SMAD7 ASO;

b) about 20% to about 50% by weight mannitol;

c) about 10% to about 30% by weight microcrystalline cellulose,

d) about 0.5% to about 10% by weight hydroxypropyl methylcellulose;

e) about 0.5% to about 10% by weight sodium starch glycolate;

f) about 0.05% to about 1% by weight magnesium stearate;

g) about 0.5% to about 10% by weight Opadry® AMB; and

h) about 5% to about 20% by weight Acryl-EZE®.

21. The pharmaceutical composition of any one of claims 1-18, comprising:

a) about 8.5% by weight of the SMAD7 ASO;

b) about 40% by weight mannitol;

c) about 25% by weight microcrystalline cellulose,

d) about 5% by weight hydroxypropyl methylcellulose;

e) about 4% by weight sodium starch glycolate;

f) about 0.4% by weight magnesium stearate;

g) about 4% by weight Opadry® AMB; and

h) about 10% to about 15% by weight Acryl-EZE®.

22. The pharmaceutical composition of any one of claims 1-18, comprising:

a) about 23% by weight of the SMAD7 ASO;

b) about 28% by weight mannitol;

c) about 25% by weight microcrystalline cellulose,

d) about 5% by weight hydroxypropyl methylcellulose;

e) about 4% by weight sodium starch glycolate;

f) about 0.4% by weight magnesium stearate;

g) about 4% by weight Opadry® AMB; and

h) about 7% to about 12% by weight Acryl-EZE®.

23. The pharmaceutical composition of any one of claims 1-22, wherein the pharmaceutical composition is formulated as a tablet.

24. The pharmaceutical composition of any one of claims 1-23, wherein the pharmaceutical composition down-regulates SMAD7 mRNA and/or protein in a cell.

25. The pharmaceutical composition of claim 24, wherein the down-regulation of SMAD7 protein expression is more than 40% compared to an untreated cell.

26. The pharmaceutical composition of any one of claims 1-25, wherein the pharmaceutical composition is clinically efficacious in treating or preventing inflammatory bowel disease.

27. The pharmaceutical composition of claim 26, wherein the inflammatory bowel disease is Crohn's disease.

28. The pharmaceutical composition of claim 27, wherein the Crohn's disease is postoperative recurrence of Crohn's disease.

29. The pharmaceutical composition of any one of claims 1-28 for oral administration.

30. The pharmaceutical composition of any one of claims 1-29 for oral administration, wherein the SMAD7 ASO is administered to a subject in need thereof at a dose of about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100 mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, or about 200 mg.

31. The pharmaceutical composition of any one of claims 1-30 for oral administration about every 6 hours, about every 12 hours, about every 24 hours, about every 48 hours, about every 72 hours, every day, two-times a week, once in 2 weeks, or once a month.

32. The pharmaceutical composition of any one of claims 1-31 for oral administration at a dose of about 160 mg daily.

33. A method of treating or preventing an inflammatory bowel disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of any one of claims 1-32.

34. Use of a pharmaceutical composition of any one of claims 1-32 for the manufacture of a medicament for the treatment of an inflammatory bowel disease.

35. A pharmaceutical composition of any one of claims 1-32 for use in the treatment of an inflammatory bowel disease.

36. The method of claim 33, use of claim 34, or pharmaceutical composition for use of claim 35, wherein the inflammatory bowel disease is Crohn's disease.

37. The method of claim 33 or 36, use of claim 34 or 36, or pharmaceutical composition for use of claim 35 or 36, wherein the Crohn's disease is postoperative recurrence of Crohn's disease .

38. A method of preventing or treating postoperative recurrence of Crohn's disease (CD) in a subject in need thereof, comprising inhibiting SMAD7 in the subject.

39. The method of claim 38, wherein at least one sample from the subject has an elevated SMAD7 level relative to a known control level, wherein the known control level is the SMAD7 level in a sample collected from the subject prior to or during the surgical treatment for CD or the SMAD7 level in a sample collected from a healthy subject without CD.

40. A method of preventing or treating postoperative recurrence of Crohn's disease (CD) in a subject in need thereof, comprising preventing a Crohn's Disease Activity Index (CDAI) score greater than 200 of the subject or reducing CDAI score for at least 50 points.

41. The method of claim 40, comprising preventing a CDAI score greater than 150.

42. The method of claim 40, comprising reducing CDAI score for at least 100 points.

43. A method for predicting postoperative recurrence of Crohn's disease (CD) in a subject in need thereof, comprising determining the level of SMAD7 in a first sample from the subject, wherein an elevated SMAD7 level relative to a known control level is predictive of the recurrence of CD, wherein the known control level is the SMAD7 level in a sample collected from the subject prior to or during the surgical treatment for CD or the SMAD7 level in a sample collected from a healthy subject without CD.

44. A method of identifying a subject at risk of postoperative recurrence of Crohn's disease (CD), comprising: determining the level of SMAD7 in a first sample from the subject, wherein an elevated SMAD7 level relative to a known control level identifies the subject as being at risk for the recurrence of CD, wherein the known control level is the SMAD7 level in a sample collected from the subject prior to or during the surgical treatment for CD or the SMAD7 level in a sample collected from a healthy subject without CD.

45. The method of claim 43 or 44, comprising:

a) if the SMAD7 level is elevated relative to the known control level, then administering to the subject a pharmaceutical composition comprising a SMAD7 ASO; or

b) if the SMAD7 level is not elevated relative to the known control level, then determining the level of SMAD7 in a second sample from the subject.

46. The method of claim 45, wherein the second sample is collected immediately after, about 1 hour after, about 3 hours after, about 6 hours after, about 12 hours after, about 1 day after, about 3 days after, about 1 week after, about 2 weeks after, about 1 month after, about 2 months after, about 3 months after, about 4 months after, about 5 months after, about 6 months after, about 7 months after, about 8 months after, about 9 months after, about 10 months after, about 11 months after, or about 12 months after the first sample.

47. A method of preventing or treating postoperative recurrence of Crohn's disease (CD) in a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising a SMAD7 ASO.

48. The method of claim 38, wherein inhibiting SMAD7 in the subject comprises administering to the subject a pharmaceutical composition comprising a SMAD7 ASO.

49. The method of claim 47 or 48, wherein the SMAD7 ASO is administered at a dose of about 40 mg, about 50 mg, about 60 mg, about 70 mg, about 80 mg, about 90 mg, about 100

mg, about 110 mg, about 120 mg, about 130 mg, about 140 mg, about 150 mg, about 160 mg, about 170 mg, about 180 mg, about 190 mg, or about 200 mg.

50. The method of any one of claims 47-49, wherein the SMAD7 ASO is administered about every 6 hours, about every 12 hours, about every 24 hours, about every 48 hours, about every 72 hours, every day, two-times a week, once in 2 weeks, or once a month.

51. The method of any one of claims 47-50, wherein the SMAD7 ASO is administered at a dose of about 160 mg daily.

52. The method of any one of claims 47-51, wherein the SMAD7 ASO comprises a sequence selected from any one of SEQ ID NOs: 1-6 or a pharmaceutically acceptable salt thereof.

53. The method of any one of claims 47-52, wherein the SMAD7 ASO has a sequence according to SEQ ID NO: 1 (5'-GTXGCCCCTTCTCCCXGCAGC-3') in which all internucleoside linkages are *O,O*-linked phosphorothioate linkages and X is 5-methyl 2'-deoxycytidine.

54. The method of any one of claims 47-53, wherein the pharmaceutical composition comprises a plurality of diastereomers of a SMAD7 ASO having a sequence according to SEQ ID NO: 1 (5'-GTXGCCCCTTCTCCCXGCAGC-3') in which all internucleoside linkages are *O,O*-linked phosphorothioate linkages and X is 5-methyl 2'-deoxycytidine,
wherein the plurality of diastereomers has a ^{31}P-NMR spectrum comprising:

a) one or more resonances between 54.8 to 55.5 ppm; and

b) a first principal component 1 (PC1) score and a second principal component 2 (PC2) score, wherein:

(i) the PC1 score is below about -0.20 or above about 0.25 and the PC2 score is below about 0.00; or

(ii) the PC1 score is outside of the range of -0.32 to 0.30 and/or the PC2 score is outside the range of 0.00 to 0.20.

55. The method of claim 54, wherein the ^{31}P-NMR spectrum comprises:

a) a PC1 score below about -0.20 and a PC2 score below about 0.00;

b) a PC1 score above about 0.25 and a PC2 score below about 0.00;

c) a PC1 score from about -0.47 to about -0.20 and a PC2 score from about -0.27 to about 0.00;

d) a PC1 score from about 0.65 to about 0.9 and a PC2 score from about -0.47 to about 0.00;

e) a PC1 score outside of the range of -0.32 to 0.30 when the PC2 score is above 0.00 and below 0.20; and/or

f) a PC2 score outside the range of 0.00 to 0.20 when the PC1 is above -0.32 and below 0.30.

56. The method of any one of claims 38-55, wherein the postoperative recurrence is selected from the group consisting of: endoscopic recurrence, histological recurrence, radiographic recurrence, clinical recurrence, and combinations thereof.

57. The method of any one of claims 38-56, wherein the subject has received at least one surgical treatment for CD.

58. The method of any one of claims 57, wherein the at least one surgical treatment is selected from a group consisting of: bowel resection, ileocolonic resection, colectomy, proctocolectomy, strictureplasty, ileostomy, anal fistulotomy, and combinations thereof.

59. The method of claim 58, wherein the at least one surgical treatment is ileocolonic resection.

60. The method of any one of claims 38-59, wherein the subject exhibits no sign of postoperative recurrence.

61. The method of any one of claims 38-60, wherein the subject exhibits at least one sign of postoperative recurrence.

62. The method of claim 61, wherein the subject exhibits at least one sign of endoscopic recurrence.

63. The method of claim 62, wherein the at least one sign is a mucosal lesion.

64. The method of any one of claims 39-63, wherein the sample is a mucosal sample.

65. The method of claim 39-64, wherein the sample is collected immediately after, about 1 hour after, about 3 hours after, about 6 hours after, about 12 hours after, about 1 day after, about 3 days after, about 1 week after, about 2 weeks after, about 1 month after, about 2 months after, about 3 months after, about 4 months after, about 5 months after, about 6 months after, about 7 months after, about 8 months after, about 9 months after, about 10 months after, about 11 months after, or about 12 months after a surgical treatment for CD.

66. The method of any one of claims 39-65, wherein the SMAD7 level is SMAD7 mRNA level or SMAD7 protein level.

67. The method of any one of claims 39-66, wherein the elevated SMAD7 level is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 60%, or at least about 80% elevated as compared to the known control level.

68. The method of any one of claims 45-67, wherein the pharmaceutical composition is administered orally.

69. The method of any one of claims 45-68, wherein the pharmaceutical composition is administered immediately after, about 1 hour after, about 3 hours after, about 6 hours after, about 12 hours after, about 1 day after, about 3 days after, about 1 week after, about 2 weeks after, about 1 month after, about 2 months after, about 3 months after, about 4 months after, about 5 months after, about 6 months after, about 7 months after, about 8 months after, about 9 months after, about 10 months after, about 11 months after, or about 12 months after a surgical treatment for CD.

70. A pharmaceutical composition comprising a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO) that is pharmacologically and/or clinically efficacious, made by a method comprising:

a) confirming a ^{31}P-NMR spectrum for the plurality of diastereomers of one or more resonances between 54.8 to 55.5 ppm; and

b) confirming the ^{31}P-NMR spectrum has:

(i) a first principal component 1 (PC1) score that is below about -0.20 or above about 0.25 and a second principal component 2 (PC2) score that is below about 0.00, or

(ii) a PC1 score that is outside of the range of -0.32 to 0.30 and/or a PC2 score that is outside the range of 0.00 to 0.20,

as determined by principal component analysis (PCA).

71. A method of manufacturing a pharmaceutical composition comprising a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO), the method comprising:

a) confirming a ^{31}P-NMR spectrum for the plurality of diastereomers of one or more resonances between 54.8 to 55.5 ppm; and

b) confirming the ^{31}P-NMR spectrum has:

(i) a first principal component 1 (PC1) score that is below about -0.20 or above about 0.25 and a second principal component 2 (PC2) score that is below about 0.00, or

(ii) a PC1 score that is outside of the range of -0.32 to 0.30 and/or a PC2 score that is outside the range of 0.00 to 0.20,

as determined by principal component analysis (PCA).

72. The pharmaceutical composition of claim 70 or method of claim 71, wherein the method comprises confirming:

a) the PC1 score is below about -0.20 and the PC2 score is below about 0.00;

b) the PC1 score is above about 0.25 and the PC2 score is below about 0.00;

c) the PC1 score is from about -0.47 to about -0.20 and the PC2 score is from about -0.27 to about 0.00;

d) the PC1 score is from about 0.65 to about 0.9 and the PC2 score is from about -0.47 to about 0.00;

e) the PC1 score is outside of the range of -0.32 to 0.30 when the PC2 score is above 0.00 and below 0.20; and/or

f) the PC2 score is outside the range of 0.00 to 0.20 when the PC1 is above -0.32 and below 0.30.

73. The pharmaceutical composition of claim 70 or 72 or method of claim 71 or 72, wherein the method comprises confirming:

a) the ^{31}P-NMR spectrum has two or more resonances between 54.8 to 55.5 ppm;

b) the ^{31}P-NMR spectrum has one or more resonances at about 54.8 ppm;

c) the ^{31}P-NMR spectrum has two or more resonances at about 54.8 ppm;

d) the ^{31}P-NMR spectrum has one or more resonances at about 54.9 ppm;

e) the ^{31}P-NMR spectrum has two or more resonances at about 54.9 ppm;

f) the ^{31}P-NMR spectrum has one or more resonances at about 55.0 ppm;

g) the ^{31}P-NMR spectrum has two or more resonances at about 55.0 ppm;

h) the ^{31}P-NMR spectrum has one or more resonances at about 55.1 ppm;

i) the ^{31}P-NMR spectrum has two or more resonances at about 55.1 ppm;

j) the ^{31}P-NMR spectrum has one or more resonances at about 55.2 ppm;

k) the ^{31}P-NMR spectrum has two or more resonances at about 55.2 ppm;

l) the ^{31}P-NMR spectrum has one or more resonances at about 55.3 ppm;

m) the ^{31}P-NMR spectrum has two or more resonances at about 55.3 ppm;

n) the ^{31}P-NMR spectrum has one or more resonances at about 55.4 ppm;

o) the ^{31}P-NMR spectrum has two or more resonances at about 55.4 ppm;

p) the ^{31}P-NMR spectrum has one or more resonances at about 55.5 ppm;

q) the ^{31}P-NMR spectrum has two or more resonances at about 55.5 ppm; and/or

r) the ^{31}P-NMR spectrum has two or more resonances between 54.8 to 55.5 with different intensities.

74. A method of predicting pharmacological and/or clinical efficacy of a candidate composition comprising a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO), the method comprising subjecting a ^{31}P-NMR spectrum for the plurality of diastereomers to principal component analysis (PCA) to obtain a first principal component (PC1) and a second principal component (PC2), wherein:

a) a PC1 score that is below about -0.20 or above about 0.25 and a PC2 score that is below about 0.00 is predictive of pharmacological and/or clinical efficacy, or

b) a PC1 score that is outside of the range of -0.32 to 0.30 and/or a PC2 score that is outside the range of 0.00 to 0.20,

is predictive of pharmacological and/or clinical efficacy.

75. The method of claim 74, wherein:

a) a PC1 score that is below about -0.20 and a PC2 score that is below about 0.00;

b) a PC1 score above about 0.25 and a PC2 score that is below about 0.00;

c) a PC1 score from about -0.47 to about -0.20 and a PC2 score that is from about -0.27 to about 0.00;

d) a PC1 score from about 0.65 to about 0.9 and a PC2 score that is from about -0.47 to about 0.00;

e) a PC1 score outside of the range of -0.32 to 0.30 when the PC2 score is above 0.00 and below 0.20; and/or

f) a PC2 score is outside the range of 0.00 to 0.20 when the PC1 is above -0.32 and below 0.30,

is predictive of pharmacological and/or clinical efficacy.

76. The pharmaceutical composition of claim 70 or 72 or method of any one of claims 71-75, wherein the PCA comprises selecting principle components using a modeling data set.

77. The pharmaceutical composition of claim 76 or method of claim 76, wherein the modeling data set comprises five, six, seven, eight, nine, ten, or up to twenty-seven ^{31}P-NMR spectra selected from the ^{31}P-NMR spectra as shown in FIGs. 5C-5CC.

78. The pharmaceutical composition of claim 76 or 77 or method of claim 76 or 77, wherein the modeling data set comprises the ^{31}P-NMR spectra as shown in FIGs. 5C, 5L, 5X, 5H, 5P, 5I, 5M, 5T, 5S, and 5BB.

79. The pharmaceutical composition of any one of claims 70 and 71-78 or method of any one of claims 71-78, wherein the principal components are selected to account for more than 90% variance of the modeling data set.

80. The pharmaceutical composition of any one of claims 70 and 71-79 or method of any one of claims 71-79, wherein the ^{31}P-NMR is performed in solution at about 14.1 T.

81. The pharmaceutical composition of any one of claims 70 and 71-80 or method of any one of claims 71-80, wherein the SMAD7 ASO comprises a sequence selected from any one of SEQ ID NOs: 1-6 or a pharmaceutically acceptable salt thereof.

82. The pharmaceutical composition of any one of claims 70 and 71-81 or method of any one of claims 71-81, wherein internucleotide linkages of the SMAD7 ASO are *O,O*-linked phosphorothioates.

83. The pharmaceutical composition of any one of claims 70 and 71-82 or method of any one of claims 71-82, wherein the SMAD7 ASO has a sequence according to SEQ ID NO: 1 (5'-GTXGCCCCTTCTCCCXGCAGC-3') in which all internucleoside linkages are *O,O*-linked phosphorothioate linkages and X is 5-methyl 2'-deoxycytidine.

84. A method of treating an inflammatory bowel disease in a subject in need thereof, comprising administering to the subject the pharmaceutical composition of any one of claims 70 and 71-83, the SMAD7 ASO manufactured according to any one of claims 71-73 and 76-83 or the SMAD7 ASO predicted to be pharmacologically and/or clinically efficacious according to any one of claims 74-83.

85. The method of claim 84, wherein the inflammatory bowel disease is Crohn's disease.

86. The method of claim 85, wherein the Crohn's disease is postoperative Crohn's disease.