

[0011] There remains a need for strategies for predicting, treating and preventing postoperative recurrence of CD. The present disclosure provides methods for predicting, treating, and/or preventing postoperative recurrence of CD and for identifying patients at risk of postoperative recurrence of CD.

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SUMMARY

[0012] The present application describes pharmaceutical compositions of SMAD7 antisense oligonucleotide diastereomers and methods of manufacturing, assessing efficacy, and using the compositions.

10 **[0013]** In one aspect, the present disclosure refers to a pharmaceutical composition, comprising a SMAD7 antisense oligonucleotide (ASO), wherein the SMAD7 ASO consists of 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'-
15 deoxycytidine, wherein the plurality of diastereomers has a phosphorus-31 nuclear magnetic resonance (31P-NMR) spectrum consisting of: a) one or more resonances between 54.8 to 55.5 ppm; b) a first principal component 1 (PC1) score and a second principal component 2 (PC2) score, as determined by principal component analysis (PCA), wherein: i. the PC1 score is below -0.32 or above 0.25 and the PC2 score is below about 0.00; ii. the PC1 score is below -0.32 and the PC2 score is below 0.00; or iii the PC1 score is from 0.65 to 0.9 and the PC2 score is from
20 0.47 to 0.00.

[0013a] In another aspect, the present disclosure refers to the pharmaceutical composition as described herein for use in therapy, wherein the pharmaceutical composition is to be orally administered.

25 **[0013b]** Accordingly, one example of the present application is 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
30 magnetic resonance (³¹P-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.

- 5 **[0014]** In some examples, 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. In some examples, 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. In some examples, 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. In some examples, the ^{31}P -
10 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. In some examples, 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. In some examples, 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.
- 15 **[0015]** In some examples, the ^{31}P -NMR spectrum comprises two or more resonances between 54.8 to 55.5 ppm. In some examples, the two or more resonances between 54.8 to 55.5 ppm have different intensities. In some examples, 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
20 resonances. In some examples, the ^{31}P -NMR spectrum comprises, independently at about

pharmaceutical composition comprises: 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®.

[0019] In some examples, the pharmaceutical composition is formulated as a tablet. In some examples, the pharmaceutical composition is for oral administration. In some examples, the pharmaceutical composition is 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. In some examples, the pharmaceutical composition is 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. In some examples, the pharmaceutical composition is for oral administration at a dose of about 160 mg daily.

[0020] In some examples, the pharmaceutical composition down-regulates SMAD7 mRNA and/or protein in a cell. In some examples, the down-regulation of SMAD7 protein expression is more than 40% compared to an untreated cell. In some examples, the pharmaceutical composition is clinically efficacious in treating or preventing inflammatory bowel disease. In some examples, the inflammatory bowel disease is Crohn's disease. In some examples, the Crohn's disease is postoperative recurrence of Crohn's disease.

[0021] In one example, the present disclosure refers to a method of treating or preventing an inflammatory bowel disease in a subject in need thereof, comprising administering to the subject a pharmaceutical composition described herein. In one aspect, the present disclosure refers to a use of a pharmaceutical composition described herein in the manufacture of a medicament for the treatment of an inflammatory bowel disease. In one aspect, the present disclosure refers to a pharmaceutical composition described herein for use in the treatment of an inflammatory bowel disease. In some examples, the inflammatory bowel disease is Crohn's disease. pharmaceutical composition, the Crohn's disease is postoperative recurrence of Crohn's disease

[0022] In another example, the present disclosure describes 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. In some examples, 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.

[0023] In another example, the present disclosure describes a method for preventing or treating postoperative recurrence of Crohn's disease (CD) in a subject in need thereof,

5 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. In some examples, the method comprises preventing a CDAI score greater than 150. In some examples, the method comprises reducing CDAI score for at least 100 points.

[0024] In another example, the present disclosure describes a method for predicting
10 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
15 without CD.

[0025] In another example, the present disclosure describes 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
20 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.

[0026] In some examples, if the SMAD7 level is elevated relative to the known control level, then administering to the subject a pharmaceutical composition comprising a SMAD7
25 ASO; or 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.

[0027] In some examples, 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
30 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.

[0028] In another example, the present disclosure describes a method for preventing or treating postoperative recurrence of Crohn's disease (CD) in a subject in need thereof, comprising
35 administering to the subject a pharmaceutical composition comprising a SMAD7 ASO.

strictureplasty, ileostomy, anal fistulotomy, and combinations thereof. In some examples, the at least one surgical treatment is ileocolonic resection.

[0033] In some examples, the subject exhibits no sign of postoperative recurrence. In some examples, the subject exhibits at least one sign of postoperative recurrence. In some examples, the subject exhibits at least one sign of endoscopic recurrence. In some examples, the at least one sign is a mucosal lesion.

[0034] In some examples, the sample is a mucosal sample. In some examples, 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.

[0035] In some examples, the SMAD7 level is SMAD7 mRNA level or SMAD7 protein level. In some examples, 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.

[0036] In some examples, the pharmaceutical composition is administered orally. In some examples, 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.

[0037] In another example, the present disclosure describes 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 ³¹P-NMR spectrum for the plurality of diastereomers of one or more resonances between 54.8 to 55.5 ppm; and b) confirming the ³¹P-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).

[0038] In another aspect, the present disclosure refers to a method of manufacturing a pharmaceutical composition comprising a plurality of diastereomers of a SMAD7 antisense

oligonucleotide (ASO), the method comprising: a) producing a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO) having a sequence according to SEQ ID NO: 1 (5'-GTXGCCCCCTTCTCCCXGCAGC-3') in which all internucleoside linkages are O,O-linked phosphorothioate linkages and X is 5-methyl 2'-deoxycytidine; b) confirming a ³¹P-NMR spectrum for the plurality of diastereomers of one or more resonances between 54.8 to 55.5 ppm; and c) confirming the ³¹P-NMR spectrum has: (i) a first principal component 1 (PC1) score that is below -0.32 or above 0.25 and a second principal component 2 (PC2) score that is below about 0.00, (ii) a PC1 score that is below -0.32 and a PC2 score that is below 0.00; or (iii) a PC1 score that is from 0.65 to 0.9 and a PC2 score that is from -0.47 to 0.00, as determined by principal component analysis (PCA).

[0039] In some examples, 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. In some examples, the method comprises confirming: a) the ³¹P-NMR spectrum has two or more resonances between 54.8 to 55.5 ppm; b) the ³¹P-NMR spectrum has one or more resonances at about 54.8 ppm; c) the ³¹P-NMR spectrum has two or more resonances at about 54.8 ppm; d) the ³¹P-NMR spectrum has one or more resonances at about 54.9 ppm; e) the ³¹P-NMR spectrum has two or more resonances at about 54.9 ppm; f) the ³¹P-NMR spectrum has one or more resonances at about 55.0 ppm; g) the ³¹P-NMR spectrum has two or more resonances at about 55.0 ppm; h) the ³¹P-NMR spectrum has one or more resonances at about 55.1 ppm; i) the ³¹P-NMR spectrum has two or more resonances at about 55.1 ppm; j) the ³¹P-NMR spectrum has one or more resonances at about 55.2 ppm; k) the ³¹P-NMR spectrum has two or more resonances at about 55.2 ppm; l) the ³¹P-NMR spectrum has one or more resonances at about 55.3 ppm; m) the ³¹P-NMR spectrum has two or more resonances at about 55.3 ppm; n) the ³¹P-NMR spectrum has one or more resonances at about 55.4 ppm; o) the ³¹P-NMR spectrum has two or more resonances at about 55.4 ppm; p) the ³¹P-NMR spectrum has one or more resonances at about 55.5 ppm; q) the ³¹P-NMR spectrum has two or more resonances at about 55.5 ppm; and/or r) the ³¹P-NMR spectrum has two or more resonances between 54.8 to 55.5 with different intensities.

[0040] In another example, the present disclosure describes 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
5 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) the PC1 score is outside of the range of -0.32 to 0.31 and/or the PC2 score that is outside the range of 0.00 to 0.20, is predictive of pharmacological and/or clinical efficacy. In some examples, 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) the 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.

[0041] In some examples, the PCA comprises selecting principle components using a modeling data set. In some examples, the modeling data set comprises five, six, seven, eight, nine, ten, or up to twenty-seven ³¹P-NMR spectra selected from the ³¹P-NMR spectra as shown in FIGs. 5C-5CC. In some examples, the modeling data set comprises the ³¹P-NMR spectra as shown in FIGs. 5C, 5L, 5X, 5H, 5P, 5I, 5M, 5T, 5S, and 5BB. In some examples, the principal components are selected to account for more than 90% variance of the modeling data set. In some examples, the ³¹P-NMR is performed in solution at about 14.1 T. In some examples, the SMAD7 ASO comprises a sequence selected from any one of SEQ ID NOs: 1-6 or a pharmaceutically acceptable salt thereof. In some examples, internucleotide linkages of the SMAD7 ASO are *O,O*-linked phosphorothioates. In some examples, the SMAD7 ASO has a sequence according to SEQ ID NO: 1 (5'-GTXGCCCTTCTCCCXGCAGC-3') in which all internucleoside linkages are *O,O*-linked phosphorothioate linkages and X is 5-methyl 2'-deoxycytidine.

[0042] In some examples, described here is a method of treating an inflammatory bowel disease in a subject in need thereof, comprising administering to the subject a SMAD7 ASO manufactured according a method disclosed herein or a SMAD7 ASO predicted to be pharmacologically and/or clinically efficacious according a method disclosed herein. In some examples, the inflammatory bowel disease is Crohn's disease. In some examples, the Crohn's disease is postoperative Crohn's disease.

[0043] These and other aspects and advantages and other aspects of disclosure described in the present application are illustrated by the following figures, detailed description, and claims.

CLAIMS

WHAT IS CLAIMED IS:

1. A pharmaceutical composition, comprising a SMAD7 antisense oligonucleotide (ASO), wherein the SMAD7 ASO consists of a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO) having a sequence according to SEQ ID NO: 1 (5'-GTXGCCCCCTTCTCCCXGCAGC-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 (31P-NMR) spectrum consisting of:
 - 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, as determined by principal component analysis (PCA), wherein:
 - i. the PC1 score is below -0.32 or above 0.25 and the PC2 score is below about 0.00;
 - ii. the PC1 score is below -0.32 and the PC2 score is below 0.00; or
 - iii. the PC1 score is from 0.65 to 0.9 and the PC2 score is from 0.47 to 0.00
2. The pharmaceutical composition of claim 1, wherein one or more cytidines are a 5-methyl 2'-deoxycytidine.
3. The pharmaceutical composition of claim 1, wherein the 31P-NMR spectrum comprises two or more resonances between 54.8 to 55.5 ppm.
4. The pharmaceutical composition of claim 3, wherein the two or more resonances between 54.8 to 55.5 ppm have different intensities.
5. The pharmaceutical composition of any one of claims 1-4, wherein the 31P-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.

6. The pharmaceutical composition of any one of claims 1-5, 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.
7. The pharmaceutical composition of any one of claims 1-6, comprising:
 - a) 0.5% to 30% by weight of the SMAD7 ASO;
 - b) 20% to 50% by weight mannitol;
 - c) 10% to 30% by weight microcrystalline cellulose; and
 - d) an enteric coating comprising an ethylacrylate-methacrylic acid copolymer.
8. The pharmaceutical composition of any one of claims 1-6, comprising:
 - a) an intra-granular phase comprising:
 - i. 5% to 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.

9. The pharmaceutical composition of any one of claims 1-6, comprising:

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

b) 20% to 50% by weight mannitol;

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

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

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

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

g) 5% to 20% by weight ethylacrylate-methacrylic acid copolymers.

10. The pharmaceutical composition of any one of claims 1-6, 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; and

g) 10% to 15% by weight ethylacrylate-methacrylic acid copolymers.

11. The pharmaceutical composition of any one of claims 1-6, 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; and
 - g) 7% to 12% by weight ethylacrylate-methacrylic acid copolymers.
12. The pharmaceutical composition of any one of claims 1-11, wherein the pharmaceutical composition is formulated as a tablet.
13. The pharmaceutical composition of any one of claims 1-12 for use in therapy, wherein the pharmaceutical composition is to be orally administered.
14. The pharmaceutical composition of any one of claims 1-13, wherein the SMAD7 ASO is to be orally 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.
15. The pharmaceutical composition of any one of claims 1-14, wherein the pharmaceutical composition is to be orally 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.
16. The pharmaceutical composition of any one of claims 1-15, wherein the pharmaceutical composition is to be orally administered at a dose of about 160 mg daily.

17. Use of a pharmaceutical composition of any one of claims 1-16 in the manufacture of a medicament for the treatment of an inflammatory bowel disease.
18. A pharmaceutical composition of any one of claims 1-16 for use in the treatment of an inflammatory bowel disease.
19. The use of claim 17, or the pharmaceutical composition of claim 18, wherein the inflammatory bowel disease is Crohn's disease.
20. The use of claim 17, or the pharmaceutical composition of claim 18, wherein the Crohn's disease is postoperative recurrence of Crohn's disease.
21. A method of manufacturing a pharmaceutical composition comprising a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO), the method comprising:
- a) producing a plurality of diastereomers of a SMAD7 antisense oligonucleotide (ASO) having a sequence according to SEQ ID NO: 1 (5'-GTXGCCCCCTTCTCCCXGCAGC-3') in which all internucleoside linkages are O,O-linked phosphorothioate linkages and X is 5-methyl 2'-deoxycytidine;
 - b) confirming a ³¹P-NMR spectrum for the plurality of diastereomers of one or more resonances between 54.8 to 55.5 ppm; and
 - c) confirming the ³¹P-NMR spectrum has:
 - i. a first principal component 1 (PC1) score that is below -0.32 or above 0.25 and a second principal component 2 (PC2) score that is below 0.00,
 - ii. a PC1 score that is below -0.32 and a PC2 score that is below 0.00; or
 - iii. a PC1 score that is from 0.65 to 0.9 and a PC2 score that is from -0.47 to 0.00,
- as determined by principal component analysis (PCA).