

CLAIMS

1. A method for diagnosing or monitoring a non-alcoholic fatty liver disease (NAFLD), non-
5 alcoholic steatohepatitis (NASH) and/or liver fibrosis, and/or for determining the efficacy of a
treatment of a NAFLD, NASH and/or liver fibrosis, comprising determining the level of miR-
193 in a body fluid sample of said subject.
2. The method according to claim 1, wherein miR-193 is hsa-miR-193, in particular hsa-miR-
10 193a-3p, hsa-miR-193a-5p, hsa-miR-193b-5p or hsa-miR-193b-3p.
3. The method according to claim 1 or 2, comprising determining the level of hsa-miR-193b-
3p.
- 15 4. The method according to any one of claims 1 to 3, wherein the body fluid sample is a
sample of blood, plasma or serum.
5. The method according to any one of claims 1 to 4, wherein the level of miR-193 is
compared to reference levels of miR-193.
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6. The method according to claim 5, wherein a level of miR-193 that is higher than a
reference levels obtained in samples from healthy subjects with no hepatic steatosis
indicates the presence of NAFLD.
- 25 7. The method according to claim 5, wherein a level of miR-193 that is higher than reference
range levels obtained in samples from a non-NASH subject indicates the presence of NASH
defined as at least one point in steatosis, lobular inflammation and hepatocyte ballooning
score.
- 30 8. The method according to claim 5, wherein a level of miR-193 that is higher than a
reference range level obtained in samples from subjects without Active-NASH indicates the
presence of Active-NASH.
9. The method according to claim 5, wherein a level of miR-193 that is higher than reference
35 levels obtained in samples from subjects with no or minimal liver fibrosis (F=0 or 1) indicates
the presence of a significant (F=2), moderate (F=3) or severe (F=4) liver fibrosis.

10. The method according to any one of the preceding claims, comprising the classification of a subject as being potential receiver (TBT) or non-receiver (NTBT) of a treatment for NASH and/or liver fibrosis, based on the detection of an increased level of miR-193 relative to a reference level of miR-193 measured in NTBT subjects.

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11. The method according to claim 10, wherein TBT and NTBT subjects have the following liver biopsy-derived grades:

a) TBT1:

steatosis score ≥ 1

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hepatocyte ballooning score ≥ 1

lobular inflammation score ≥ 1

NAS (NAFLD Activity Score) ≥ 4

fibrosis stage ≥ 1 (such as a fibrosis stage equal to 1, 2, 3 or 4 3)

NTBT1:

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differs from TBT1 in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage;

or

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b) TBT2:

steatosis score ≥ 1

hepatocyte ballooning score ≥ 1

lobular inflammation score ≥ 1

NAS (NAFLD Activity Score) ≥ 4

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fibrosis stage ≥ 2 (such as a fibrosis stage equal to 2, 3 or 4, in particular 2 or 3)

NTBT2:

differs from TBT in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage;

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or

c) TBT7:

steatosis score ≥ 1

hepatocyte ballooning score ≥ 1

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lobular inflammation score ≥ 1

NAS (NAFLD Activity Score) ≥ 4

fibrosis stage fibrosis stage = 1b, 1c, 2, 3 or 4

NTBT2:

differs from TBT in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage.

- 5 12. The method according to any one of the preceding claims, comprising monitoring the evolution of NAFLD activity, NASH activity or liver fibrosis in the subject based on the evolution of the level of miR-193 in samples collected two or more times apart from the same subject.
- 10 13. The method according to any one of claims 1 to 13, for evaluating the efficiency of a treatment of NAFLD, NASH or liver fibrosis, based on the evolution of the level of miR-193 in samples collected two or more times apart from the same treated subject.
- 15 14. An anti-NAFLD, anti-NASH or anti-fibrosis compound for use in a method for treating a subject having NAFLD, NASH or liver fibrosis respectively, wherein the subject is diagnosed or classified as a to-be-treated patient because of NAFLD, NASH or liver fibrosis according to any one of claims 1 to 11, and said compound is to be administered to said diagnosed subject.
- 20 15. A miR-193 inhibitory compound for use in a method for treating a subject having NAFLD, NASH and/or liver fibrosis.
- 25 16. The miR-193 inhibitory compound for use according to claim 15, wherein the subject was diagnosed or classified to-be-treated according to any one of claims 1 to 11.
- 30 17. Use of an anti-NAFLD, anti-NASH or anti-fibrosis compound in the preparation of a medicament for treating a subject having NAFLD, NASH or liver fibrosis respectively, wherein the subject is diagnosed or classified as a to-be-treated patient because of NAFLD, NASH or liver fibrosis according to any one of claims 1 to 11.
- 35 18. Use of a miR-193 inhibitory compound in the preparation of a medicament for treating a subject having NAFLD/NASH and/or liver fibrosis.
19. The use according to claim 18, wherein the subject was diagnosed or classified to-be-treated according to any one of claims 1 to 11.

20. A method for the diagnosis of non-alcoholic steatohepatitis (NASH) and/or for determining the activity, the stage, or the severity of NASH in a subject, and/or for the classification of a subject as a receiver or non receiver of a treatment for NASH, and/or for the evaluation of the efficacy of a medical treatment, and/or for the determination of the progression or the regression of the pathology in NASH patients, and/or for the classification of a patient as a potential responder or non responder to a medical treatment, and/or for the prediction of disease outcome for a patient, and/or for the identification of surrogate markers of clinical relevant outcomes, comprising the measure of the level of blood, serum or plasma circulating hsa-miR-193 and at least one other blood, serum or plasma circulating marker of liver damage.

21. The method according to claim 20, for the diagnosis of NASH or for the classification of a subject as a receiver or non-receiver of a treatment for NASH.

22. The method according to claim 20 or 21, wherein said at least one other circulating marker of liver damage is selected in the group consisting of TIMP-1, YKL-40, platelet count, HbA1c and Hyaluronic acid.

23. The method according to claim 22, further comprising determining the metabolic syndrome score of the subject.

24. The method according any one of claims 20 to 23, comprising the steps of:

- measuring the level of hsa-miR-193, (in particular hsa-miR-193b and more particularly hsa-miR-193b-3p), and at least one blood, serum or plasma circulating marker of liver damage, and
- combining the results through a mathematical algorithm to obtain a NASH score.

25. The method according to claim 23, wherein a NASH score is calculated according to the following logistic function:

$$S1 = \frac{e^{Y1}}{1 + e^{Y1}}$$

wherein:

$$Y1 = k + a \cdot A + b \cdot B + c \cdot C + d \cdot D + f \cdot F$$

wherein:

S1 is the NASH score;

A is the level of miR-193 in log10 copies.μL⁻¹;

B is the level of TIMP-1 in ng/mL;

C is the level of YKL-40 in pg/mL;

D is the platelet count in $10^9/L$;

F is the level of metabolic syndrome in category;

5 k is the constant of the logistic function;

a is a coefficient associated to the level of miR-193 (in particular hsa-miR-193b-3p);

b is a coefficient associated to the level of TIMP-1;

c is a coefficient associated to the level of YKL-40;

10 d is a coefficient associated to the platelet count and;

f is a coefficient associated to the metabolic syndrome

the method is derived from the bootstrap model, wherein:

k is a number comprised between -8.39 and -2.67, in particular -5.53.

15 a is a number comprised between 0.58 and 2.50, in particular 1.54

b is a number comprised between $2.29E-03$ and $1.87E-02$, in particular $1.05E-02$

c is a number comprised between $2.14E-06$ and $1.65E-05$, in particular $9.34E-06$

d is a number comprised between $-1.56E-02$ and $-1.05E-03$, in particular $-8.32E-03$

f is a number comprised between 0.03 and 1.63, in particular 0.83

20 and wherein the threshold value is comprised between 0.3050 and 0.6518 in particular 0.3993.

wherein a NASH score higher than a threshold value comprised between 0.2017 and 0.4645, in particular equal to 0.2302, is indicative of a severe NASH, or of a moderate or high NASH

25 activity; and thus is indicative of a patient having a steatosis score ≥ 1 , a hepatocyte ballooning score ≥ 1 , a lobular inflammation score ≥ 1 , a NAS ≥ 4 and a fibrosis stage ≥ 2 .

26. The method according to claim 23, wherein the NASH score is calculated according to the following logistic function:

$$S2 \sim \frac{e^{Y2}}{1 + e^{Y2}}$$

30 wherein:

$$Y2 = l + a2 \cdot A + c2 \cdot C + b2 \cdot B + e \cdot E + g \cdot G + d2 \cdot D$$

wherein:

S2 is the NASH score;

A is the level of hsa-miR-193 in $\log_{10}$ copies. μL^{-1} ;

35 C is the level of YKL-40 in pg/mL;

B is the level of TIMP-1 in ng/mL;

E is the level of HbA1c in percent;

G is the level of Hyaluronic Acid in ng/mL;

D is the platelet count: $10^9/L$;

5 I is the constant of the logistic function;

a2 is a coefficient associated to the level of miR-193 (in particular hsa-miR-193b-3p);

c2 is a coefficient associated to the the level of YKL-40;

b2 is a coefficient associated to the level of TIMP-1;

10 e is a coefficient associated to the level of HbA1c

g is a coefficient associated to the level of Hyaluronic Acid

d2 is a coefficient associated to the platelet count.

the method is derived from the bootstratp model, wherein:

15 I is a number comprised between -10.92 and -3.89, in particular -7.52

a2 is a number comprised between 0.44 and 2.17, in particular 1.29

c2 is a number comprised between 0.0035 and 0.02, in particular 0.01

b2 is a number comprised between 0.0016 and 0.018, in particular 0.0092

e is a number comprised between -0.0053 and 0.0057, in particular -0.0024

20 g is a number comprised between 0.13 and 1.08, in particular 0.59

d2 is a number comprised between -0.017 and -0.0027, in particular -0.009

and wherein the threshold value is comprised between 0.2461 and 0.6252 in particular 0.4216.

25 wherein a NASH score higher than a threshold value comprised between 0.2461 and 0.6252, in particular equal to 0.4216 is indicative of a severe NAHS, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1 , a hepatocyte ballooning score ≥ 1 , a lobular inflammation score ≥ 1 , a NAS ≥ 4 and a fibrosis stage ≥ 2 .

30 27. The method according to claim 23, wherein the NASH score is calculated according to the following logistic function:

$$S3 \sim \frac{e^{Y3}}{1 + e^{Y3}}$$

wherein:

$$Y3 = m + a3 \cdot A' + c3 \cdot C + b3 \cdot B + e2 \cdot E + g2 \cdot G + d3 \cdot D$$

35 wherein:

S3 is the NASH score according to the present invention;

A' is the level of hsa-miR-193b-3p in Cq;

C is the level of YKL-40 in pg/mL;

B is the level of TIMP-1 in ng/mL;

5 E is the level of HbA1c in percent;

G is the level of Hyaluronic Acid in ng/mL;

D is the platelet count: $10^9/L$;

m is the constant of the logistic function;

10 a3 is a coefficient associated to the level of miR-193 (in particular hsa-miR-193b-3p);

c3 is a coefficient associated to the the level of YKL-40;

b3 is a coefficient associated to the level of TIMP-1;

e2 is a coefficient associated to the level of HbA1c

g2 is a coefficient associated to the level of Hyaluronic Acid

15 d3 is a coefficient associated to the platelet count.

The method is derived from the bootstrap model wherein:

m is a number comprised between -1.27 and 17.54, in particular 8.08

a3 is a number comprised between -0.64 and -0.09 in particular -0.38

20 c3 is a number comprised between 0.005 and 0.019, in particular 0.01

b3 is a number comprised between 0.001 and 0.016, in particular 0.01

e2 is a number comprised between -0.0054 and 0.0045, in particular 0.0024

g2 is a number comprised between 0.12 and 1.08, in particular 0.57

d3 is a number comprised between -0.0164 and -0.0016, in particular -0.0090

25 and wherein the threshold value is comprised between 0.2270 and 0.6364 in particular 0.3741.

wherein a NASH score higher than a threshold value comprised between 0.2270 and 0.6364,

in particular equal to 0.3741 is indicative of a severe NAHS, or of a moderate or high NASH

30 activity; and thus is indicative of a patient having a steatosis score ≥ 1 , a hepatocyte ballooning score ≥ 1 , a lobular inflammation score ≥ 1 , a NAS ≥ 4 and a fibrosis stage ≥ 2 .

28. A kit comprising means for determining the level of:

(i) at least one marker selected in the group consisting of hsa-miR-193, (in particular hsa-

35 miR-193b and more particularly hsa-miR-193b-3p), and

(ii) and at least one blood, serum or plasma circulating marker of liver damage.

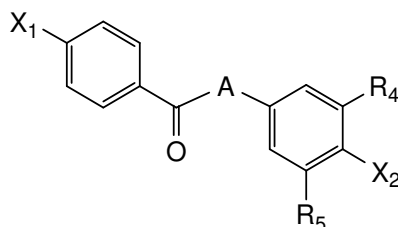
29. The kit according to claim 28, comprising means for determining the level of:

(i) at least one marker selected in the group consisting of hsa-miR-193, (in particular hsa-miR-193b and more particularly hsa-miR-193b-3p); and

(ii) and at least one blood, serum or plasma circulating marker of liver damage corresponding used in the method or use according to any one of claims 1 to 28.

30. An anti-NASH molecule for use in a method for the treatment of NASH, wherein said anti-NASH molecule is to be administered to a patient in need thereof diagnosed with NASH or classified according to the method of any one of claims 1 to 11, in particular classified as receiver of a treatment for NASH.

31. The anti-NASH molecule for use according to claim 30, wherein said molecule is of formula (I):



wherein:

X1 represents a halogen, a R1, or G1-R1 group;

A represents a CH=CH or a CH2-CH2 group;

X2 represents a G2-R2 group;

G1 and G2, identical or different, represent an atom of oxygen or sulfur;

R1 represents a hydrogen atom, an unsubstituted alkyl group, an aryl group or an alkyl group that is substituted by one or more halogen atoms, an alkoxy or an alkylthio group, cycloalkyl groups, cycloalkylthio groups or heterocyclic groups;

R2 represents an alkyl group substituted by at least a -COOR3 group, wherein R3 represents a hydrogen atom, or an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, or heterocyclic groups.

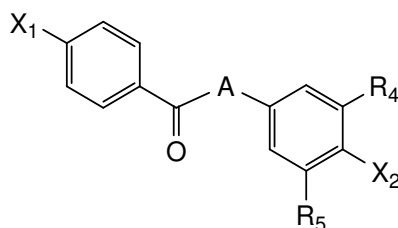
R4 and R5, identical or different, representing an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, heterocyclic groups; or a pharmaceutically acceptable salt thereof.

32. The anti-NASH molecule for use according to claim 30 or 31, wherein the compound is selected in the group consisting of 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxy phenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-isopropoxy carbonyldimethylmethoxyphenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-

[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethoxyphenyl] prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyl dimethylmethoxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethoxyphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethoxy phenyl] prop-2-en-1-one, 1-[4-trifluoromethoxyphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxyphenyl]prop-2-en-1-one, 2-[2,6-dimethyl-4-[3-[4-(methylthio)phenyl]-3-oxo-propyl] phenoxy]-2-methylpropanoic acid, and 2-[2,6-dimethyl-4-[3-[4-(methylthio) phenyl]-3-oxo-propyl]phenoxy]-2-methylpropanoic acid isopropyl ester; or a pharmaceutically acceptable salt thereof, in particular 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxy phenyl]prop-2-en-1-one or a pharmaceutically acceptable salt thereof.

33. Use of an anti-NASH molecule in the preparation of a medicament for the treatment of NASH, wherein said anti-NASH molecule is to be administered to a patient in need thereof diagnosed with NASH or classified according to the method of any one of claims 1 to 11, in particular classified as receiver of a treatment for NASH.

34. The use according to claim 33, wherein the anti-NASH molecule is of formula (I):



wherein:

X1 represents a halogen, a R1, or G1-R1 group;

A represents a CH=CH or a CH2-CH2 group;

X2 represents a G2-R2 group;

G1 and G2, identical or different, represent an atom of oxygen or sulfur;

R1 represents a hydrogen atom, an unsubstituted alkyl group, an aryl group or an alkyl group that is substituted by one or more halogen atoms, an alkoxy or an alkylthio group, cycloalkyl groups, cycloalkylthio groups or heterocyclic groups;

R2 represents an alkyl group substituted by at least a -COOR3 group, wherein R3 represents a hydrogen atom, or an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, or heterocyclic groups.

R4 and R5, identical or different, representing an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, heterocyclic groups; or a pharmaceutically acceptable salt thereof.

35. The use according to any one of claims 33 to 34, wherein the anti-NASH molecule is selected in the group consisting of 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxy phenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-isopropoxy carbonyldimethylmethoxyphenyl]prop-2-en-1-one, 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethoxyphenyl] prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyl dimethylmethoxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethylphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxyphenyl]prop-2-en-1-one, 1-[4-trifluoromethyl oxyphenyl]-3-[3,5-dimethyl-4-tertbutyloxycarbonyldimethylmethoxy phenyl] prop-2-en-1-one, 1-[4-trifluoromethoxyphenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxyphenyl]prop-2-en-1-one, 2-[2,6-dimethyl-4-[3-[4-(methylthio)phenyl]-3-oxo-propyl] phenoxy]-2-methylpropanoic acid, and 2-[2,6-dimethyl-4-[3-[4-(methylthio) phenyl]-3-oxo-propyl]phenoxy]-2-methylpropanoic acid isopropyl ester; or a pharmaceutically acceptable salt thereof, in particular 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxy phenyl]prop-2-en-1-one or a pharmaceutically acceptable salt thereof.