

CLAIMS

1. An *in vitro* method for diagnosing or monitoring a non-alcoholic fatty liver disease (NAFLD),
5 non-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-193b-3p in a body fluid sample of a subject, wherein the level of miR-193b-3p is
compared to reference levels of miR-193b-3p, and
- of at least one other blood, serum or plasma circulating marker of liver damage selected from
10 the group consisting of TIMP-1, YKL-40, platelet count, HbA1c and Hyaluronic acid.
2. The method according to claim 1, wherein the body fluid sample is a sample of blood, plasma
or serum.
- 15 3. The method according to claim 1 or 2, wherein a level of miR-193b-3p that is higher than ~~a~~
reference levels obtained in samples from healthy subjects with no hepatic steatosis indicates
the presence of NAFLD.
- 20 4. The method according to claim 1 or 2, wherein a level of miR-193b-3p 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.
- 25 5. The method according to claim 1 or 2, wherein a level of miR-193b-3p that is higher than a
reference range level obtained in samples from subjects without Active-NASH indicates the
presence of Active-NASH.
- 30 6. The method according to claim 1 or 2, wherein a level of miR-193b-3p that is higher than
reference 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.
- 35 7. The method according to claim 1 or 2 ~~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-
193b-3p relative to a reference level of miR-193b-3p measured in NTBT subjects.
8. The method according to claim 7, wherein TBT and NTBT subjects have the following liver
biopsy-derived grades:

a) TBT1:steatosis score ≥ 1 hepatocyte ballooning score ≥ 1 lobular inflammation score ≥ 1 5 NAS (NAFLD Activity Score) ≥ 4 fibrosis stage ≥ 1 (such as a fibrosis stage equal to 1, 2, 3 or 4 ~~3~~)NTBT1:

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;

10

or

b) TBT2:steatosis score ≥ 1 15 hepatocyte ballooning score ≥ 1 lobular inflammation score ≥ 1 NAS (NAFLD Activity Score) ≥ 4 fibrosis stage ≥ 2 (such as a fibrosis stage equal to 2, 3 or 4, in particular 2 or 3)NTBT2:20 differs from TBT~~2~~ in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage;

or

25 c) TBT7:steatosis score ≥ 1 hepatocyte ballooning score ≥ 1 lobular inflammation score ≥ 1 NAS (NAFLD Activity Score) ≥ 4

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

NTBT~~7~~2:differs from TBT~~7~~ in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage.

35 9. An *in vitro* method for diagnosing or monitoring a non-alcoholic fatty liver disease (NAFLD), non-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-193b-3p in a body fluid sample of a subject, wherein the level of miR-193b-3p is compared to reference levels of miR-193b-3p, said method 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-193b-3p relative to a reference level of miR-193b-3p measured in NTBT subjects,

wherein TBT and NTBT subjects have the following liver biopsy-derived grades:

a) TBT1:

steatosis score ≥ 1

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)

NTBT1:

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

b) TBT2:

steatosis score ≥ 1

hepatocyte ballooning score ≥ 1

lobular inflammation score ≥ 1

NAS (NAFLD Activity Score) ≥ 4

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

NTBT2:

differs from TBT2 in a at least one point lesser grade in any of steatosis score,

hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage;

or

c) TBT7:

steatosis score ≥ 1

hepatocyte ballooning score ≥ 1

lobular inflammation score ≥ 1

NAS (NAFLD Activity Score) ≥ 4

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

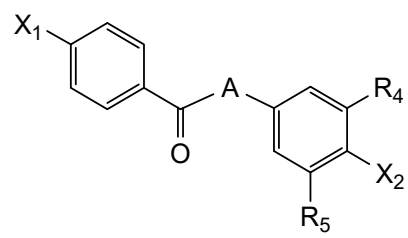
NTBT7:

differs from TBT7 in a at least one point lesser grade in any of steatosis score, hepatocyte ballooning score, lobular inflammation score, NAS or fibrosis stage.

109. 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-193b-3p in samples collected two or more times apart from the same subject.

1140. The method according to any one of claims 1 to 98, for evaluating the efficiency of a treatment of NAFLD, NASH or liver fibrosis, based on the evolution of the level of miR-193b-3p in samples collected two or more times apart from the same treated subject.

1244. 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 according to any one of claims 1 to 6, or 9 or classified as a to-be-treated patient because of NAFLD, NASH or liver fibrosis according to any one of claims 74 to 98, wherein the 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 represents an atom of oxygen;

G2 represents 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.

5 1312. Use of a miR-193b-3p inhibitory compound in the preparation of a medicament for treating a subject having NAFLD/NASH and/or liver fibrosis, wherein the miR-193b-3p inhibitory compound is selected from the group consisting of short- or small-interfering RNA ("siRNA"), antagomirs and antisense nucleic acids.

10 1413. The use according to claim 1312, wherein the subject was diagnosed according to any one of claims 1 to 6 or 9 or classified to-be-treated according to any one of claims 7 to 91 to 8.

15 ~~14. An *in vitro* 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~~
20 ~~of clinical relevant outcomes, comprising the measure of the level of blood, serum or plasma circulating hsa-miR-193b-3p and at least one other blood, serum or plasma circulating marker of liver damage, wherein the level of miR-193b-3p is compared to reference levels of miR-193b-3p.~~

25 15. The method according to claim 1 or 244, for the diagnosis of NASH or for the classification of a subject as a receiver or non-receiver of a treatment for NASH.

~~16. The method according to claim 14 or 15, 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.~~
30

1617. The method according to claim 1 or 246, further comprising determining the metabolic syndrome score of the subject.

35 1718. The method according any one of claims 144 to 1617, comprising the steps of:
- measuring the level of 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.

1849. The method according to claim 17, wherein a NASH score is calculated according to the following logistic function:

5

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

wherein:

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

wherein:

10 S1 is the NASH score;

A is the level of miR-193b-3p 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 10⁹/L;

15 F is the level of metabolic syndrome in category;

k is the constant of the logistic function;

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

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

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

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:

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

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

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

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

35 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.

1920. The method according to claim 17, wherein the NASH score is calculated according to the following logistic function:

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

5 wherein:

$$Y2 = I + a2 * A + c2 * C + b2 * B + e * E + g * G + d2 * D$$

wherein:

S2 is the NASH score;

A is the level of hsa-miR-193b-3p in log10 copies. μL^{-1} ;

10 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: $10^9/\text{L}$;

15 I is the constant of the logistic function;

a2 is a coefficient associated to the level of 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;

20 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:

25 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

30 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.

wherein a NASH score higher than a threshold value comprised between 0.2461 and 0.6252,

35 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 .

2024. The method according to claim 17, wherein the NASH score is calculated according to the following logistic function:

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

wherein:

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

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;

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;

a3 is a coefficient associated to the level of hsa-miR-193b-3p;

c3 is a coefficient associated to 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

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

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

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 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 .

5

~~2122.~~ A kit comprising means for determining the level of:

(i) hsa-miR-193b-3p, and

(ii) and at least one blood, serum or plasma circulating marker of liver damage selected from the group consisting of TIMP-1, YKL-40, platelet count, HbA1c and Hyaluronic acid,

10 wherein the kit comprises as means antibodies specific for a protein to be quantified and primers for quantifying micro-RNA levels.

~~23. The kit according to claim 22, comprising means for determining the level of:~~

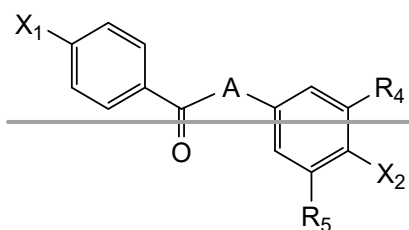
~~(i) hsa-miR-193b-3p; and~~

15 ~~(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 23.~~

~~24. 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~~

20 ~~diagnosed with NASH or classified according to the method of any one of claims 1 to 8, in particular classified as receiver of a treatment for NASH.~~

~~25. The use according to claim 24, wherein the anti-NASH molecule is of formula (I):~~



25 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;~~

30 ~~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.

26. The use according to any one of claims 24 to 25, wherein the anti-NASH molecule is selected in the group consisting of 1-[4-methylthiophenyl]-3-[3,5-dimethyl-4-carboxydimethylmethoxyphenyl]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-tertbutyloxycarbonyldimethylmethoxyphenyl]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-tertbutyloxycarbonyldimethylmethoxyphenyl]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-carboxydimethylmethoxyphenyl]prop-2-en-1-one or a pharmaceutically acceptable salt thereof.