

CLAIMS

1. A method for the diagnosis of non-alcoholic steatohepatitis (NASH) and/or for determining
5 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
10 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-34 or one of its collinear variable, and at least one other blood, serum or plasma
circulating marker of liver damage.
- 15 2. The method according to claim 1, for the diagnosis of NASH or for the classification of a
subject as a receiver or non-receiver of a treatment for NASH.
3. The method according to claim 1 or 2, wherein said at least one other circulating marker of
liver damage is selected in the group consisting of alpha 2 macroglobulin, glycated
20 haemoglobin (HbA1c), fasting glucose level, fructosamine level, insulin, C-Peptide,
Homeostasis Model Assessment (HOMA), N-terminal pro-peptide of collagen type III, hsa-
miR-200, YKL-40, CK18-M30, CK18-M65, ALT, AST, Urinary Specific Gravity (Uri Spec
Grav), urinary creatinine, basophils, High Sensitivity-Reactive Protein (HSCRP), Urinary β -
NAG, leucocytes, neutrophils and fibrinogen;
- 25 wherein the method may further comprising measure of at least one other parameter such as
the height of the subject.
4. The method according any one of claims 1 to 3, comprising the steps of:
- measuring the level of hsa-miR-34, (in particular hsa-miR-34a and more particularly hsa-
30 miR-34a-5a), 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.
5. The method according to claim 4, wherein a NASH score is calculated according to the
35 following logistic function:

$$S = \frac{e^Y}{1 + e^Y}$$

wherein:

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

wherein:

- 5 S is the NASH score;
- A is the level of alpha 2 macroglobulin in g/L;
- B is the level of HbA1c in percent;
- C is the level of N-terminal pro-peptide of collagen type III in ng/mL;
- D is the level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;
- 10 F is the level of hsa-miR-200 (in particular c hsa-miR-200a, more particularly hsa-miR-200a-3p) in Cq;
- G is the level of YKL-40 in pg/ml.
- k is the constant of the logistic function;
- a is a coefficient associated to the level of alpha 2 macroglobulin;
- 15 b is a coefficient associated to the level of HbA1c;
- c is a coefficient associated to the level of N-terminal pro-peptide of collagen type III;
- d is a coefficient associated to the level of hsa-miR-34a (in particular hsa-miR-34a-5p);
- f is a coefficient associated to the level of hsa-miR-200 (in particular hsa-miR-200a, more particularly hsa-miR-200a-3p); and
- 20 g is a coefficient associated to the level of YKL-40;

wherein either:

- a) the method is derived from the median model, and:
 - 25 k is a number comprised between 5.94 and 50.74, in particular 25.13;
 - a is a number comprised between 0 and 1.07, in particular 0.52;
 - b is a number comprised between 0 and 1.20, in particular 0.57;
 - c is a number comprised between 0 and 0.24, in particular 0.12;
 - d is a number comprised between -0.97 and 0, in particular -0.43;
 - 30 f is a number comprised between -0.87 and 0, in particular -0.47;
 - g is a number comprised between 0 and 1.74E-05, in particular 9.54E-06;

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

or wherein:

b) the method is derived from the bootstrap model, wherein:

k is a number comprised between 8.24 and 35.44, in particular 21.84;

5 a is a number comprised between 0.06 and 0.88, in particular 0.47;

b is a number comprised between 0.14 and 1.04, in particular 0.59;

c is a number comprised between 0.03 and 0.23, in particular 0.13;

d is a number comprised between -0.75 and -0.05, in particular -0.4;

f is a number comprised between -0.73 and -0.07, in particular -0.4; and

10 g is a number comprised between 3.59E-06 and 1.78E-05, in particular 1.07E-05;

wherein a NASH score higher than a threshold value comprised between 0.2718 and 0.6391, in particular equal to 0.3502, is indicative of a severe NASH, 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 .

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6. The method according to claim 4, wherein a NASH score is calculated according to the following logistic function:

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$$S \sim \frac{e^Y}{1 + e^Y}$$

wherein:

$$Y = k + a \cdot A + b \cdot B + c \cdot C + d \cdot D$$

wherein either:

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a) the method is derived from the bootstrap model, and wherein

S is the NASH score;

A is the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

B is the serum level of alpha 2 macroglobulin in g/L;

30 C is the serum level of YKL-40 in pg/ml,

D is the level of HbA1c in percent (e.g. D is equal to 10 if measured HbA1c percentage is 10%);

k is the constant of the logistic function

35 a is a coefficient associated to the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p);

b is a coefficient associated to the serum level of alpha 2 macroglobulin;

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

d is a coefficient associated to the level of HbA1c;

wherein:

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k is a number comprised between 9.51 and 34.37, in particular 21.94;

a is a number comprised between -1.17 and -0.47, in particular -0.82;

b is a number comprised between 0.02 and 0.84, in particular 0.43;

c is a number comprised between 6.10E-06 and 2.09E-05, in particular 1.35E-05;

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d is a number comprised between 0.07 and 0.89, in particular 0.48;

wherein a NASH score higher than a threshold value comprised between 0.2013 and 0.5965, in particular equal to 0.4661, is indicative of a severe NASH, 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 ;

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or wherein:

b) the method is derived from the median model, wherein:

k is a number comprised between 6.02 and 56.69, in particular 28.17;

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a is a number comprised between -1.26 and 0.00, in particular -0.84;

b is a number comprised between 0.00 and 0.88, in particular 0.36,

c is a number comprised between 0.00 and 2.00E-05, in particular 1.23E-05;

d is a number comprised between 0.00 and 0.96 in particular 0.41;

25 wherein a NASH score higher than a threshold value comprised between 0.9773 and 0.9955 is indicative of a severe NASH, 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 7. The method according to claim 4, wherein a NASH score is calculated according to the following logistic function:

$$S \sim \frac{e^Y}{1 + e^Y}$$

wherein:

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$$Y = k + a \cdot A + b \cdot B + c \cdot C + d \cdot D$$

wherein:

S is the NASH score;

A is the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p) in copy/ μ L (log10);

B is the serum level of alpha 2 macroglobulin in g/L;

C is the serum level of YKL-40 in pg/ml,

5 D is the level of HbA1c in percent (e.g. D is equal to 10 if measured HbA1c percentage is 10%);

k is the constant of the logistic function

a is a coefficient associated to the serum level of hsa-miR-34a (in particular hsa-miR-34a-5p);

10 b is a coefficient associated to the serum level of alpha 2 macroglobulin;

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

d is a coefficient associated to the level of HbA1c;

wherein either:

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a) the method is derived from the bootstrap model, wherein:

k is a number comprised between -14.50 and -7.40, in particular -10.95;

a is a number comprised between 1.38 and 3.58, in particular 2.48;

b is a number comprised between 0.02 and 0.84, in particular 0.43;

20 c is a number comprised between 5.98E-06 and 2.08E-05, in particular 1.34E-05;

d is a number comprised between 0.07 and 0.89, in particular 0.48;

wherein a NASH score higher than a threshold value comprised between 0.1895 and 0.6089, in particular equal to 0.4255, is indicative of a severe NASH, 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 ;

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or wherein:

b) the method is derived from the median model, wherein:

30 k is a number comprised between -27.16 and -0.78, in particular -11.03;

a is a number comprised between 0 and 3.97, in particular 2.59;

b is a number comprised between 0 and 0.89, in particular 0.38,

c is a number comprised between 0 and 1.98E-05, in particular 1.21E-05;

d is a number comprised between 0 and 0.97, in particular 0.40;

35 wherein a NASH score higher than a threshold value comprised between 0.1421 and 0.4556, in particular equal to 0.3201, is indicative of a severe NASH, 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 .

8. The method according to claim 4, wherein a NASH score is calculated according to the following logistic function:

$$S \sim \frac{e^Y}{1 + e^Y}$$

wherein:

$$Y = k + a \cdot A + b \cdot B$$

- 10 wherein:

S is the NASH score;

A is the level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

B is the level of YKL-40 in pg/ml;

k is a number comprised between -116.08 and 40.97, in particular 12.87;

- 15 a is a number comprised between -0.82 and 0, in particular -0.42;

b is a number comprised between 0 and 1.88E-05, in particular 8.160E-06;

wherein a NASH score higher than a threshold value comprised between 0.1387 and 0.3481, in particular equal to 0.2187, is indicative of a severe NASH, 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 ≥ 1 .

9. The method according to claim 4, wherein a NASH score is calculated according to the following logistic function:

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$$S \sim \frac{e^Y}{1 + e^Y}$$

wherein:

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

wherein:

- 30 S is the NASH score;

A is the level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

B is the level of YKL-40 in pg/ml;

C is the level of urinary specific gravity (no units);

D is the level of basophils in 10e9/L;

- 35 F is the level of HSCRP in mg/dL

k is a number comprised between -124.81 and 1.13, in particular -61.84

a is a number comprised between -0.91 and -0.25, in particular -0.58;

b is a number comprised between 4.77e-06 and 2.39e-05, in particular 1.44E-05;

c is a number comprised between 17.21 and 141.51, in particular 79.36;

5 d is a number comprised between 2.80 and 60.74, in particular 31.77;

f is a number comprised between 0.01 and 0.23, in particular 0.12;

wherein a NASH score higher than a threshold value comprised between 0.5791 and 0.8269, in particular equal to 0.7758, is indicative of a severe NASH, or of a moderate or high NASH activity; and thus is indicative of a patient having a steatosis score ≥ 1 , a hepatocyte
10 ballooning score ≥ 1 , a lobular inflammation score ≥ 1 , a NAS ≥ 4 and a fibrosis stage ≥ 1 .

10. The method according to claim 4, wherein a NASH score is calculated according to the following logistic function:

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$$S = \frac{e^Y}{1 + e^Y}$$

wherein:

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

wherein:

S is the NASH score;

20 A is the level of in particular hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

B is the level of CK18-M65 in U/L;

C is the level of Urinary β -NAG in U/L;

D is the level of Leukocytes in 10e9/L;

F is the level alpha 2 macroglobulin in g/L;

25 G is the level of has-miR-200a (in particular hsa-miR-200a-3p) in Cq,

k is a number comprised between -69.89 and 57.02, in particular 24.98;

a is a number comprised between -1.09 and 0, in particular -0.54;

b is a number comprised between 0 and 0.005, in particular 0.002;

c is a number comprised between 0 and 0.28, in particular 0.14;

30 d is a number comprised between 0 and 0.69, in particular 0.32;

f is a number comprised between 0 and 1.08, in particular 0.43;

g is a number comprised between -0.80 and 0, in particular -0.37;

wherein a NASH score higher than a threshold value comprised between 0.0197 and 0.1136, in particular equal to -0.37, 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 = 1b, 1c, 2, 3 or 4.

11. The method according to claim 4, wherein a NASH score is calculated according to the following logistic function:

$$S = \frac{e^Y}{1 + e^Y}$$

wherein:

$$Y = k + a \cdot A + b \cdot B + c \cdot C + d \cdot D + f \cdot F + g \cdot G + h \cdot H$$

wherein:

S is the NASH score;

A is the level of hsa-miR-34a (in particular hsa-miR-34a-5p) in Cq;

B is the level of CK18-M65 in U/L

15 C is the level of Urinary β -NAG in U/L

D is the level of Leukocytes in 10E9/L

F is the level alpha 2 macroglobulin in g/L

G is the level of fibrinogen in g/L,

H is the level of the height in cm,

20 k is a number comprised between 8.31 and 35.79, in particular 22.05;

a is a number comprised between -0.96 and -0.26, in particular -0.61;

b is a number comprised between 0.0006 and 0.0034, in particular 0.002;

c is a number comprised between 0.04 and 0.24, in particular 0.14;

d is a number comprised between 0.04 and 0.52, in particular 0.28;

25 f is a number comprised between 0.07 and 0.89, in particular 0.48;

g is a number comprised between 0.14 and 1.28, in particular 0.71;

h is a number comprised between -0.08 and -0.001, in particular -0.04;

wherein a NASH score higher than a threshold value comprised between 0.3373 and 0.7260, in particular equal to 0.6370, is indicative of a severe NASH, 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 = 1b, 1c, 2, 3 or 4.

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

- (i) at least one marker selected in the group consisting of hsa-miR-34, (in particular hsa-miR-34a and more particularly hsa-miR-34a-5p), hsa-miR-122 (in particular hsa-miR-122-5p) and hsa-miR-192 (in particular hsa-miR-192-5p); and
- (ii) and at least one blood, serum or plasma circulating marker of liver damage.

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13. The kit according to claim 12, comprising means for determining the level of:

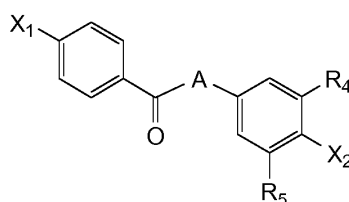
- (i) at least one marker selected in the group consisting of hsa-miR-34, (in particular hsa-miR-34a and more particularly hsa-miR-34a-5p), hsa-miR-122 (in particular hsa-miR-122-5p) and hsa-miR-192 (in particular hsa-miR-192-5p); and

- 10 (ii) and at least one blood, serum or plasma circulating marker of liver damage corresponding used in the method according to any one of claims 1 to 50.

14. An anti-NASH molecule for use in a method for the treatment of NASH, wherein said anti-NASH molecule is administered to a patient in need thereof diagnosed with NASH or

15 classified according to the method of any one of claims 1 to 50, in particular classified as receiver of a treatment for NASH.

15. The anti-NASH molecule for use according to claim 14, wherein said molecule is of formula (I):



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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;

- 25 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

30 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.

16. The anti-NASH molecule for use according to claim 14 or 15, 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-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.