CLAIMS

1. 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-452 in a body fluid sample of a subject, wherein the level of miR-452 is compared to reference levels of miR-452 and wherein the body fluid sample is a sample of blood, plasma or serum, wherein a level of miR-452 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, and wherein a level of miR-452 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 ~~wherein a level of miR-452 that is higher than reference levels obtained in samples from healthy subjects with no hepatic steatosis indicates the presence of NAFLD, or NASH~~.

2. The method according to claim 1, wherein miR-452 is hsa-miR-452, in particular hsa-miR-452-5p, hsa-miR-452-3p.

3. The method according to claim 1 or 2, comprising determining the level of hsa-miR-452-5p.

~~4. The method according to claim 1, wherein a level of miR-452 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.~~

4~~5~~. The method according to claim 1, wherein a level of miR-452 that is higher than a reference range level obtained in samples from subjects without Active-NASH indicates the presence of Active-NASH defined as NAS ≥ 4 with at least one point in steatosis, one point in lobular inflammation and one point in the hepatocyte ballooning scores.

~~6. The method according to claim 1, wherein a level of miR-452 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.~~

5~~7~~. 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-452 relative to a reference level of miR-452 measured in NTBT subjects.

68. The method according to claim 57, 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 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;

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

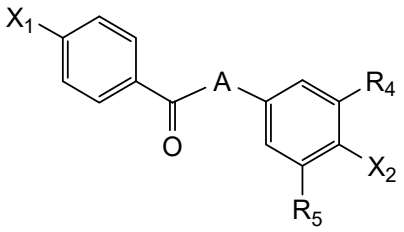
c) TBT7:
steatosis score ≥ 1
hepatocyte ballooning score ≥ 1
lobular inflammation score ≥ 1
NAS (NAFLD Activity Score) ≥ 4
fibrosis stage fibrosis stage = 1b, 1c, 2, 3 or 4
NTBT7:

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.

79. 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-452 in samples collected two or more times apart from the same subject.

810. The method according to any one of claims 1 to 79, for evaluating the efficiency of a treatment of ~~NAFLD,~~ NASH or liver fibrosis, based on the evolution of the level of miR-452 in samples collected two or more times apart from the same treated subject.

911. 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 ~~46~~, 79 and 810 or classified as a to-be-treated patient because of NASH or liver fibrosis according to claim 5 and ~~67 to 10~~, wherein said compound 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 represent 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.

10~~12~~. Use of a miR-452 inhibitory compound chosen ~~in~~from the list including short- or small-interfering RNA ("siRNA") and antagomirs which target miR-452, in the preparation of a medicament for treating a subject having ~~NAFLD,~~NASH and/or liver fibrosis~~.~~

~~13. Use of miR-452 inhibitory compound according to claim 12~~, wherein the subject was diagnosed according to any one of claims 1 to ~~4~~6, ~~7~~9 and ~~8~~10 or classified to-be-treated according to claim 5 and 6~~7 to 10~~.

11~~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,~~,~~ comprising

(i) the measure of the level of blood, serum or plasma circulating hsa-miR-452 and at least one other blood, serum or plasma circulating marker of liver damage,

(ii) combining these measures for generating mathematical models (algorithms) through bioinformatic approaches for obtaining a ~~NAFLD, ~~NASH ~~and/or liver fibrosis~~ score with high diagnostic/monitoring/prognostic/predictive performances for assessment of ~~NALFD, ~~NASH~~,~~ or Active-NASH ~~or liver fibrosis~~ in a subject.

12~~15~~. The method according to claim 11~~14~~, for the diagnosis of NASH or for the classification of a subject as a receiver or non-receiver of a treatment for NASH.

13~~16~~. A kit when used in the *in vitro* diagnosis of NASH and/or liver fibrosis, consisting of~~comprising~~ means for determining the level of:

(i) hsa-miR-452-5p, and

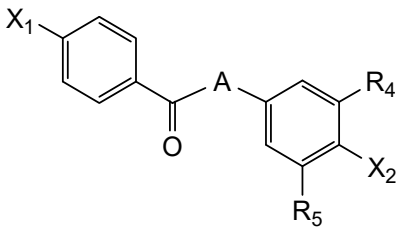
(ii) at least one biomarker selected from the group consisting of: alpha 2 macroglobulin (A2M), ~~glycated haemoglobin (HbA1c), fasting glucose level, fructosamine level, ~~N-terminal pro-peptide of collagen type III (PIIINP) and YKL-40,

wherein the kit comprises as means the antibodies specific for ~~a protein~~A2M, PIIINP and YKL-40 to be quantified, and the primers useful for quantifying hsa-miR-452-5p ~~micro-RNA ~~level~~s~~.

~~17. The kit according to claim 16, for use in the method according to any one of claims 1 to 10.~~

1418. 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 according to the method of any one of claims 1 to 46, 79, 810, 1114 and 1215 or classified according to any one of claims 7 to 105 and 6, 1114 and 1215.

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 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;

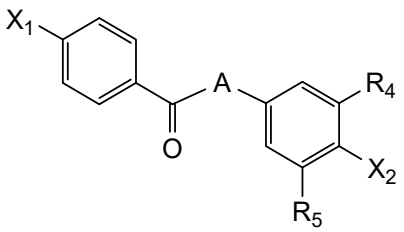
R2 represents an alkyl group substituted by at least a –COOR3 group, wherein R3 represents a hydrogen atom, or an alkyl group that is substituted or not by one or more halogen atoms, cycloalkyl groups, or heterocyclic groups.

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.

1519. Use of an anti-fibrotic molecule in the preparation of a medicament for the treatment of liver fibrosis, wherein said anti-fibrotic molecule is to be administered to a patient in need thereof diagnosed with liver fibrosis according to the method of any one of claims 1 to 46, 79, 810, 1114 and 1215 or classified as a to-be-treated patient or classified as a receiver of a treatment of liver fibrosis according to any one of claims 5 and 67 to 10, 1114 and 1215,

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 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;

or wherein said molecule is nitazoxanide or a pharmaceutically acceptable salt thereof.

16~~20~~. The use according to claim 15~~19~~, wherein the anti-fibrotic molecule is nitazoxanide.

17~~21~~. The use according to claim 16~~19~~, wherein nitazoxanide is combined with another anti-fibrotic molecule selected from vitamin E or pioglitazone, obeticholic acid, selonsertib, saroglitazar and cenicrivoc.