

SUMMARY

In one aspect, the present disclosure refers to an *in vitro* method of determining a subject's risk of liver dysfunction after partial liver resection said method comprising the steps of:

- 5 a) providing a blood sample from said subject before partial liver resection,
- b) determining in said sample the expression level of miR-151a and miR-192, and optionally of miR-122, and
 - i. identifying the ratios of miR-151a to miR-192, and optionally of miR-122 to miR-151a, based on the expression levels determined in b), ~~and~~ comparing said
10 expression level ratios with reference expression level ratios, and determining standard deviations between the compared expression level ratios, or
 - ii. comparing the expression levels of miR-151a, miR-192 and miR-122 with reference expression levels and determining standard deviations between the compared expression level ratios, and
 - 15 c) classifying the sample from the outcome of step i) or step ii) into one of at least two classes, wherein each class is one of the at least two categories "high-risk" of liver dysfunction after partial liver resection and "low-risk" of liver dysfunction after partial liver resection, wherein a difference of expression levels or expression level ratios of less than 1 standard deviation classifies the sample as "low-risk" and a difference of
20 expression levels or expression level ratios of more than 1.5 standard deviations classifies the sample as "high-risk", and
wherein the reference expression level is the expression level of at least one miRNA, selected from miR-151a, miR-192 and miR-122 of a healthy subject or of a subject without post-operative liver dysfunction or of a group of healthy subjects or of a group of
25 subjects without post-operative liver dysfunction.

In another aspect, the present disclosure refers to an *in vitro* method of monitoring regeneration-success of liver regeneration stimulation of a subject after partial liver resection, comprising the steps of

- a) providing a blood sample from said subject,
- 30 b) determining the expression level ratios of miR-151a to miR-192 and of miR-122 to miR-151a in said sample
- c) determining the expression level ratios of miR-151a to miR-192 and of miR-122 to miR-151a in a reference sample, wherein the reference sample is a subject's sample before subjected to partial liver resection~~an earlier sample of the subject,~~

d) comparing the expression level ratios of b) with the expression level ratios of c), measuring a decrease in the level of hsa-miR-151a-5p, and/or an increase in the level of hsa-miR-192-5p or hsa-miR-122-5p is an indicator for an increased risk of developing liver dysfunction and

5 e) classifying the sample of said subject from the outcome of the comparison of step d) into one of two categories, “regeneration-success” for reduced or no risk of developing liver dysfunction or “no regeneration-success” for increased risk of developing liver dysfunction.

10 It is the objective of the present disclosure, to provide reliable biomarkers, with high specificity and validity, for the prediction of liver dysfunction, in particular after partial liver resection and for the monitoring of liver function, in particular following partial liver resection or liver regeneration stimulation.

The problem is solved by the present disclosure.

15 The inventors have shown that expression levels of specific miRNAs are significantly altered in patients who developed liver dysfunction after partial liver resection compared to patients who did not develop liver dysfunction. Surprisingly, these changes in expression levels were shown in blood samples derived from patients before surgery and thus enable reliable prediction of development of liver dysfunction. Further, the risk of developing liver dysfunction after partial liver resection can be predicted with
20 precision even before surgery.

The present disclosure provides a selected set of miRNAs that are specifically up- or down-regulated and are thus useful as valuable biomarkers and represent a diagnostic and predictive signature applicable over a broad range of liver diseases.

25 According to the present disclosure there is provided an *in vitro* method of determining a subject's risk of liver dysfunction, specifically after partial liver resection, said method comprising the steps of:

- a) providing a sample from said subject,
- b) determining in said sample the expression level of at least one miRNA, selected from the group consisting of miR-151a, miR-192 and miR-122, and
 - 30 i. comparing the expression level(s) of b) with at least one reference expression level, or
 - ii. identifying the ratios of miR-151a to miR-192 and/or of miR-122 to miR-151a based on the expression levels determined in b), and comparing said expression level ratios with reference expression level ratios, and

classifying the sample from the outcome of the comparison of step i) or step ii) into one of at least two classes, wherein each class is one of the at least two categories “high-risk” and “low-risk”.

- 5 Specifically, the in vitro method provided herein allows determining a subject’s risk of developing liver dysfunction. Preferably, the in vitro method provided herein allows determining a subject’s risk of developing liver dysfunction after partial liver resection.

CLAIMS

1. An *in vitro* method of determining a subject's risk of liver dysfunction after partial liver resection said method comprising the steps of:

a) providing a blood sample from said subject before partial liver resection,
5 b) determining in said sample the expression level of miR-151a and miR-192, and optionally of miR-122, and

i. identifying the ratios of miR-151a to miR-192, and optionally of miR-122 to miR-151a, based on the expression levels determined in b), ~~and~~ comparing said expression level ratios with reference expression level ratios, and determining standard
10 deviations between the compared expression level ratios, or

ii. comparing the expression levels of miR-151a, miR-192 and miR-122 with reference expression levels and determining standard deviations between the compared
expression level ratios, and

c) classifying the sample from the outcome of step i) or step ii) into one of at
15 least two classes, wherein each class is one of the at least two categories "high-risk" of
liver dysfunction after partial liver resection and "low-risk" of liver dysfunction after partial
liver resection, wherein a difference of expression levels or expression level ratios of
less than 1 standard deviation classifies the sample as "low-risk" and a difference of
expression levels or expression level ratios of more than 1.5 standard deviations
20 classifies the sample as "high-risk", and
wherein the reference expression level is the expression level of at least one miRNA,
selected from miR-151a, miR-192 and miR-122 of a healthy subject or of a subject
without post-operative liver dysfunction or of a group of healthy subjects or of a group of
subjects without post-operative liver dysfunction.

2. ~~The *in vitro* method of claim 1, wherein in step (b)(i) the expression levels~~
25 ~~of miR-151a, miR-192 and miR-122 are determined.~~

~~3.2.~~ The *in vitro* method of claim 1 ~~or 2~~, comprising further classes of the categories "no-risk" and "medium-risk".

~~4. The *in vitro* method of any one of claims 1 to 3, wherein the reference~~
30 ~~expression level is the expression level of at least one miRNA, selected from the group~~
~~consisting of miR-151a, miR-192 and miR-122 of a healthy subject or of a subject without~~
~~post-operative liver dysfunction or of a group thereof.~~

5.3. The *in vitro* method of any one of claims 1 to 23, wherein reference expression level ratios are expression level ratios of miR-151a to miR-192 and of miR-122 to miR-151a of a healthy subject or of a subject without post-operative liver dysfunction or of a group of healthy subjects or of a group of subjects without post-operative liver dysfunction thereof.

6.4. An *in vitro* method of monitoring regeneration-success of liver regeneration stimulation of a subject after partial liver resection, comprising the steps of

a) providing a blood sample from said subject,
b) determining the expression level ratios of miR-151a to miR-192 and of miR-122 to miR-151a in said sample

c) determining the expression level ratios of miR-151a to miR-192 and of miR-122 to miR-151a in a reference sample, wherein the reference sample is a subject's sample before subjected to partial liver resection ~~an earlier sample of the subject~~,

d) comparing the expression level ratios of b) with the expression level ratios of c), measuring a decrease in the level of hsa-miR-151a-5p, and/or an increase in the level of hsa-miR-192-5p or hsa-miR-122-5p is an indicator for an increased risk of developing liver dysfunction and

e) classifying the sample of said subject from the outcome of the comparison of step d) into one of two categories, "regeneration-success" for reduced or no risk of developing liver dysfunction or "no regeneration-success" for increased risk of developing liver dysfunction.

7.5. The *in vitro* method of claim 46, wherein the liver regeneration stimulation is selected from the group consisting of induction of liver hypertrophy by portal vein embolization, induction of liver hypertrophy by associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), exercise intervention ("prehabilitation") to improve overall fitness, and pharmacological therapy reducing portal vein hypertension.

8.6. The *in vitro* method of any one of claims 1 to 57, wherein the subject is suffering from malignant lesions in the liver, preferably metastatic colorectal cancer, hepatocellular carcinoma or cholangiocellular carcinoma, or from benign liver tumors, hepatic cysts and/or parasites.

9.7. The *in vitro* method of any one of claims 1 to 68, wherein the blood sample is selected from the group consisting of serum, plasma, and platelet-poor plasma.

~~10.8.~~ The *in vitro* method of any one of claims 1 to ~~79~~, wherein the expression levels or expression level ratios of the sample are compared with the reference expression level or expression level ratios using a classification model.

5 ~~11.9.~~ The *in vitro* method of any one of claims 1 to ~~810~~, wherein a classification model classifies the sample of a subject from the outcome of the comparison with the reference into one of the at least two classes.

~~12.10.~~ The *in vitro* method of any one of claims ~~810~~ or ~~911~~, wherein the classification model is selected from the group consisting of logistic regression models, support vector machine models and decision tree models.

10 ~~13.11.~~ The *in vitro* method of any one of claims 1 to ~~1012~~, wherein the expression levels are determined using a method selected from the group consisting of a sequencing-based method, specifically next-generation sequencing, an array-based method and a PCR-based method, specifically a quantitative PCR-based method.