

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, or
- ii. comparing the expression levels of miR-151a, miR-192 and miR-122 with reference expression levels, and
- 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"
- 15 and "low-risk".

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,
- 20 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 an earlier sample of the subject,
- 25 d) comparing the expression level ratios of b) with the expression level ratios of c), and
- e) classifying the sample of said subject from the outcome of the comparison of step d) into one of two categories, "regeneration-success" or "no regeneration-success".

30 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.

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 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.

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
 - 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".

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.

Specifically, in a patient's sample the expression level of at least one miRNA selected from the group consisting of miR-151a, miR-192 and miR-122 is determined. Specifically, the expression level of at least two of miR-151a, miR-192 and miR-122 is determined. Specifically, the expression level of miR-151a and miR-192 is determined.

- 5 Specifically, the expression level of miR-151a and miR-122 is determined. Specifically, the expression level of miR-192 and miR-122 is determined.

According to a specific example, the expression levels of miR-151a, miR-192 and miR-122 are determined.

- 10 Specifically, the miRNAs used herein are selected from the group consisting of hsa-miR-151a-5p, hsa-miR-192-5p and hsa-miR-122-5p.

Specifically, a decreased expression level of miR-151a is indicative of increased risk of liver dysfunction. Specifically, an increased expression level of miR-122 and/or miR-192 is indicative of increased risk of liver dysfunction.

- 15 Specifically, in pre-surgical samples of patients with increased risk of liver dysfunction the expression level of miR-151a is down-regulated. Specifically, in pre-surgical samples of patients with increased risk of liver dysfunction the expression level of miR-122 and/or the expression level of miR-192 is up-regulated. Specifically, pre-surgical samples are samples provided from a patient before partial liver resection.

- 20 According to a specific example, the outcome of the comparison of step i) or step ii) can be classified into further classes of the categories "no-risk" and "medium-risk". Specifically, comparing the miRNA expression levels or expression level ratios in a subject's sample to the miRNA expression levels or expression level ratios in samples of subjects which did not develop liver dysfunction after partial liver resection or to samples of healthy subjects allows classification of a subject's sample into one of the categories "no-risk", "low-risk", "medium-risk" or "high-risk".

- 25 Specifically, a subject, whose sample is classified as belonging to the category "no risk", thus, has no risk or very low risk of developing liver dysfunction, in particular after partial liver resection. Preferably, no risk or very low risk refers to a risk of 25% or less than 25% to develop liver dysfunction. A subject, whose sample is classified as belonging to the category "low risk", thus, has a low risk of developing liver dysfunction after partial liver resection. Preferably, low risk refers to a risk of more than 25% and up to 50% to develop liver dysfunction. A subject, whose sample is classified as belonging to the category "medium risk", thus, has a medium risk of developing liver dysfunction after partial liver resection. Preferably, medium risk refers to a risk of more than 50%

classified as belonging to the category of “no-risk” is less likely to develop liver dysfunction after partial liver resection than a subject whose sample is classified as belonging to the category “low-risk”.

5 It is within the example of the present disclosure to use either a single reference sample from a healthy subject or a subject which did not develop liver dysfunction after partial liver resection or a pool of samples derived from healthy subjects or subjects which did not develop liver dysfunction after partial liver resection for comparison with the respective sample from the subject whose LD risk to be determined. Said pool can consist of 2, 3, 4, 5, 6, 7, or more samples, specifically up to 10, 100 or more than 100
10 samples from different individuals.

According to a specific example of the present disclosure, subjects classified as “low-risk” are subjected to partial liver resection, subjects classified as “medium-risk” are subjected to stimulation of liver regeneration before partial liver resection and subjects classified as “high-risk” are not subjected to partial liver resection. Specifically, subjects
15 classified as “no-risk” are also subjected to partial liver resection without prior stimulation of liver regeneration. Specifically, subjects classified as “medium-risk” are subject to partial liver resection, but first they are subjected to treatment aiming to stimulate liver regeneration. Preferably, following stimulation of liver regeneration regeneration-success is assessed and the subject’s risk of developing liver dysfunction after partial
20 liver resection is determined before the subject is subjected to partial liver resection.

Specifically, subjects classified as “medium-risk” are subjected to treatment before partial liver resection, which treatment is selected from the group consisting of neoadjuvant chemotherapy, portal vein embolization, associating liver partition and portal vein ligation for staged hepatectomy (ALPPS), exercise intervention
25 (“prehabilitation”) or pharmacological therapy reducing portal vein hypertension. Specifically, exercise intervention and/or diet changes can aid in liver regeneration by improvement of overall fitness and health. Specifically, a further option for subjects classified as medium-risk is a modified surgical strategy. Preferably, in said modified surgical strategy, the necessary size of the resected liver portion is reduced by
30 combination with thermic ablation of tumor centers.

Specifically, miRNA signatures described herein are used to determine a time-point for partial liver resection at which the patient’s risk of developing post-operative liver dysfunction is low. Specifically, samples of subjects that are classified as “medium-risk”, and thus subjected to treatment such as ALPPS before liver resection, are provided

According to a specific example, the sample is selected from the group consisting of blood, serum, plasma, specifically platelet-poor plasma, lymph, urine and saliva and biopsy probes. Specifically, the sample is cell-free blood.

5 According to a further specific example, the expression levels or expression level ratios of the sample are compared with the reference expression levels or expression level ratios using a classification model.

Specifically, 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.

10 Specifically, the classification model is selected from the group consisting of logistic regression models, support vector machine models and decision tree models.

Further described herein are clinically useful cut-offs predicting risk of liver dysfunction, in particular following partial liver resection. Specifically, a low stringency cut-off with a Probability Score of less than 0.59, specifically less than 0.58, 0.57, 0.56, 0.55, 0.54, 0.53, 0.52, 0.51 or 0.50 identifies subjects at low risk of developing liver
15 dysfunction and patients that can undergo partial liver resection with low risk. Preferably, a Probability Score of less than 0.58 ($P < 0.58$) classifies a patient as low-risk. Specifically, a stringent cut-off with a probability score of more than 0.58, specifically more than 0.59, 0.60, 0.61, 0.62, 0.63, 0.64 or 0.65 identifies patients with medium risk of developing liver dysfunction, such patients should be optimized prior to surgery.
20 Preferably, a Probability Score of more than 0.58 ($P > 0.58$) classifies a patient as medium-risk. Probability Scores of higher than 0.66, 0.67, 0.68, 0.69, 0.70, 0.71, 0.72, 0.73, 0.74 or 0.75 identify subjects as high-risk patients, who preferably should not undergo surgical partial liver resection. Preferably, a Probability Score of higher than 0.68 ($P > 0.68$) identifies subjects at high-risk of developing liver dysfunction.

25 According to a specific example, 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.

Specifically, the difference in miRNA levels is determined by quantitative or digital
30 PCR, DNA/RNA sequencing, specifically Next-Generation Sequencing, microarray, LuminexTM luminescence based nucleic acid assays, or other hybridization-based techniques.

Herein further provided is a kit-of-parts comprising

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, or

10 ii. comparing the expression levels of miR-151a, miR-192 and miR-122 with reference expression levels, and

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" and "low-risk".

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

3. 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
20 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. The *in vitro* method of any one of claims 1 to 3, wherein reference
25 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 thereof.

6. 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 an earlier sample of the subject,

5 d) comparing the expression level ratios of b) with the expression level ratios of c), and

e) classifying the sample of said subject from the outcome of the comparison of step d) into one of two categories, "regeneration-success" or "no regeneration-success".

10 7. The *in vitro* method of claim 6, 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.

15 8. The *in vitro* method of any one of claims 1 to 7, 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. The *in vitro* method of any one of claims 1 to 8, wherein the blood sample is selected from the group consisting of serum, plasma, and platelet-poor plasma.

20 10. The *in vitro* method of any one of claims 1 to 9, 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.

25 11. The *in vitro* method of any one of claims 1 to 10, 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. The *in vitro* method of any one of claims 10 or 11, wherein the classification model is selected from the group consisting of logistic regression models, support vector machine models and decision tree models.

30 13. The *in vitro* method of any one of claims 1 to 12, 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.