## PATENT COOPERATION TREATY

### PCT

**INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY**

(Chapter I of the Patent Cooperation Treaty)

(PCT Rule 44bis)

<table>
<thead>
<tr>
<th>FOR FURTHER ACTION</th>
<th>See item 4 below</th>
</tr>
</thead>
<tbody>
<tr>
<td>Applicant’s or agent’s file reference</td>
<td>42823-501001WO</td>
</tr>
<tr>
<td>International application No.</td>
<td>PCT/IB2012/000567</td>
</tr>
<tr>
<td>International filing date <em>(day/month/year)</em></td>
<td>07 February 2012 (07.02.2012)</td>
</tr>
<tr>
<td>Priority date <em>(day/month/year)</em></td>
<td>07 February 2011 (07.02.2011)</td>
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</tbody>
</table>

**See relevant information in Form PCT/ISA/237**

**Applicant**

BIOMIRNA HOLDINGS LTD.

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1. This international preliminary report on patentability (Chapter I) is issued by the International Bureau on behalf of the International Searching Authority under Rule 44 bis.1(a).

2. This REPORT consists of a total of 21 sheets, including this cover sheet.

   In the attached sheets, any reference to the written opinion of the International Searching Authority should be read as a reference to the international preliminary report on patentability (Chapter I) instead.

3. This report contains indications relating to the following items:

   - [x] Box No. I Basis of the report
   - [ ] Box No. II Priority
   - [x] Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - [x] Box No. IV Lack of unity of invention
   - [x] Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - [x] Box No. VI Certain documents cited
   - [ ] Box No. VII Certain defects in the international application
   - [x] Box No. VIII Certain observations on the international application

4. The International Bureau will communicate this report to designated Offices in accordance with Rules 44bis.3(c) and 93bis.1 but not, except where the applicant makes an express request under Article 23(2), before the expiration of 30 months from the priority date (Rule 44bis.2).

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### Date of issuance of this report

13 August 2013 (13.08.2013)

### Authorized officer

Cécile Chatel

e-mail: ro.ib@wipo.int

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The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No. +41 22 338 02 70

Form PCT/IB/373 (January 2004)
PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA220

PCT

WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY
(PCT Rule 43bis.1)

Date of mailing
(day/month/year) see form PCT/ISA210 (second sheet)

FOR FURTHER ACTION
See paragraph 2 below

Applicant's or agent's file reference
see form PCT/ISA220

International application No.
PCT/B2012/000567
International filing date (day/month/year)
07.02.2012
Priority date (day/month/year)
07.02.2011

International Patent Classification (IPC) or both national classification and IPC
INV. C12Q1/68

Applicant
BIOIMIRNA HOLDINGS LTD.

1. This opinion contains indications relating to the following items:

☒ Box No. I  Basis of the opinion
☐ Box No. II  Priority
☒ Box No. III  Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒ Box No. IV  Lack of unity of invention
☒ Box No. V  Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement
☒ Box No. VI  Certain documents cited
☐ Box No. VII  Certain defects in the international application
☒ Box No. VIII  Certain observations on the international application

2. FURTHER ACTION

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA220.

Name and mailing address of the ISA:
European Patent Office
P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040
Fax: +31 70 340 - 3016

Date of completion of this opinion
see form PCT/ISA210

Authorized Officer
Santagati, Fabio
Telephone No. +31 70 340-9924

Form PCT/ISA237 (Cover Sheet) (July 2009)
Box No. 1  Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of:
   - ☑ the international application in the language in which it was filed
   - □ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).

2. □ This opinion has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))

3. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this opinion has been established on the basis of a sequence listing filed or furnished:
   a. (means)
      - ☑ on paper
      - ☑ in electronic form
   b. (time)
      - □ in the international application as filed
      - □ together with the international application in electronic form
      - ☑ subsequently to this Authority for the purposes of search

4. ☑ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:
<table>
<thead>
<tr>
<th>Box No. III</th>
<th>Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</th>
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</thead>
<tbody>
<tr>
<td>The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of</td>
<td></td>
</tr>
<tr>
<td>☐ the entire international application</td>
<td></td>
</tr>
<tr>
<td>☑ claims Nos. 1-120(partially)</td>
<td></td>
</tr>
<tr>
<td>because:</td>
<td></td>
</tr>
<tr>
<td>☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international search <em>(specify)</em>:</td>
<td></td>
</tr>
<tr>
<td>☐ the description, claims or drawings <em>(indicate particular elements below)</em> or said claims Nos. are so unclear that no meaningful opinion could be formed <em>(specify)</em>:</td>
<td></td>
</tr>
<tr>
<td>☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed <em>(specify)</em>:</td>
<td></td>
</tr>
<tr>
<td>☑ no international search report has been established for the whole application or for said claims Nos. 1-120(partially)</td>
<td></td>
</tr>
<tr>
<td>☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:</td>
<td></td>
</tr>
<tr>
<td>☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.</td>
<td></td>
</tr>
<tr>
<td>☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.</td>
<td></td>
</tr>
<tr>
<td>☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rules 13ter.1(a) or (b).</td>
<td></td>
</tr>
<tr>
<td>☐ See Supplemental Box for further details</td>
<td></td>
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</tbody>
</table>
**WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY**

**Box No. IV  Lack of unity of invention**

1.  ☑️ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:

   ☑️ paid additional fees

   ☐ paid additional fees under protest and, where applicable, the protest fee

   ☐ paid additional fees under protest but the applicable protest fee was not paid

   ☐ not paid additional fees

2.  ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3.  This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☑️ not complied with for the following reasons:

   **see separate sheet**

4.  Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☑️ the parts relating to claims Nos. 1-120(partially)

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**Box No. V  Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1.  **Statement**

   **Novelty (N)**
   - Yes: Claims 2, 4, 5, 8, 9, 11-55, 57-63, 81, 82, 85-94, 99-102, 104, 105, 107-120(all partially)
   - No: Claims 1, 3, 6, 7, 10, 56, 64-80, 83, 84, 95-98, 103, 106(all partially)

   **Inventive step (IS)**
   - Yes: Claims
   - No: Claims 1-120(partially)

   **Industrial applicability (IA)**
   - Yes: Claims 1-120(partially)
   - No: Claims

2.  **Citations and explanations**

   **see separate sheet**

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Form PCT/ISA/237 (April 2007)
Box No. VI  Certain documents cited

1. Certain published documents (Rules 43bis.1 and 70.10)
   and/or
2. Non-written disclosures (Rules 43bis.1 and 70.9)
   see form 210

Box No. VIII  Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet
IV. Lack of unity (Continuation)

The common concept disclosed in the application can be considered as: miRNA expression/activity associated with lung cancer. However, this concept is not new in view of WO2010/099161 (D1), which discloses a method of predicting the clinical outcome of a patient diagnosed with lung cancer, comprising detecting the expression level of miR-21 in a cancer cell sample obtained from the patient and a method to treat lung cancer comprising administering a pharmaceutically-effective amount of an antisense to miR-21 (ct. 30, 31 and 64). Similarly: WO2009/052386 (D9) discloses a method for diagnosing lung cancer in a patient comprising evaluating expression of miR-221 and/or miR-195 in one or more samples from the patient and a method of treating lung cancer by contacting a lung cell with one or more nucleic acid comprising a miRNA sequence, at least 85% identical to a complement of one or more of miR-221, miR-93, or miR-195 (ct. 1, 26 and 27). Sozzi et al. (2010) (D2) discloses microRNA expression profile of CT screening detected lung cancer. Karkera et al. (2010) (D7) discloses the miR-21:miR-221 ratio as a serum-based diagnostic for non-small cell lung cancer. US2010/010072 (D5) discloses a method for treating, preventing or reducing the risk of a disease or disorder associated with aberrant expression of a pre-miRNA and/or miRNA by using an agent with a miRNA targeting moiety which regulates expression of the miRNAs or pre-miRNAs of SEQ ID NO: 1-29 (ct. 10), where the disease or disorder is lung cancer (ct. 15).

In view of this prior art, and due to the fact that the disclosed methods and products as such are conventional in the field of lung cancer associated miRNAs, the problem to be solved by the present invention can be considered as: providing alternative miRNAs suitable as molecules or targets for lung cancer diagnosis and treatment.

The solutions given by the present application are the ones listed as follows.

1. claims: 1-4, 6-11, 13, 16-21, 23-27, 29-70, 72-76, 80-109, 111-115, 117, 118 (partially)

A method comprising: determining the level of expression of miR-451 with at least one more miRNA listed in Tables Ia-le, Ila-lIf, Va-Ve or Vla-Vle. An article comprising a support having at least one reagent capable of binding with miR-451. An apparatus comprising a unit
capable of receiving said article, means for determining the level of expression of mir-451 and for calculating mir-451 related expression quotients. A pharmaceutical compound comprising at least mir-451.

2-23. claims: 1-120(partially)

Idem as invention 1 but wherein the miRNA is one of the other miRNAs of Tables la-le, Ila-IIf, Va-Ve or Vla-Vle and the pharmaceutical compound comprise said miRNA or an inhibitor thereof (dependig on whether the miRNA is under- or over-expressed in cancer with respect to the control).

Because no other technical features can be distinguished which, in view of the prior art could be regarded as special technical features in the sense of Rule 13.2 PCT, the ISA is of the opinion that there is no single inventive concept underlying the plurality of claimed inventions of the present application in the sense of rule 13.1 PCT.

Consequently, there is a lack of unity, and the methods claimed are different inventions not belonging to a common inventive concept.

V. Reasoned statement (Continuation)

1.1 CITATIONS
Reference is made to the following documents:


D3 CN 101 804 208 A (Nanjing Medical University; Second Affiliated Hospital of Nanjing Medi) 18 August 2010 (2010-08-18)


D12 WO 2009/147525 A1 (KAROLINSKA INST INNOVATIONS AB [SE]; PIVARCSI ANDOR [SE]; SONKOLY ENIK) 10 December 2009 (2009-12-10)


Invention 1 (miR-451)

(claims 1-4, 6-11, 13, 16-21, 23-27, 29-70, 72-76, 80-109, 111-115, 117, 118 all partially)

1.2 NOVELTY (Art. 33(2) PCT)

1.2.1 D1 discloses a microRNA expression profile in lung cancers from never-smokers. Examined were miR expression profiles in 28 matched pairs of lung cancer and noncancerous lung tissues from never-smokers by using the Ohio State miR microarray version 3.0. Eighteen miRs were found to be differentially expressed in cancers compared to noncancerous tissues, comprising miR-451, miR-21, miR-126, miR-486 and miR-30b (Table 2). By
using these markers, D1 discloses a method for determining the presence of or the risk for developing lung cancer (cl. 13-16), for determining poor prognosis (cl. 17-18). D1 further discloses a composition of matter comprising at least one miR and at least one additional composition, wherein the miR is selected from a group comprising miR-451 (cl. 7-8) and a method to treat an epidermal growth factor receptor mutant cancer in a patient in need of such treatment, comprising administering a pharmaceutically-effective amount of a miR expression promoting composition, wherein the miR is selected from the group comprising miR-451, miR-126, and miR-486 (cl. 44).

Therefore, claims 1, 3, 7, 10, 14, 56, 64-66, 68, 69, 72, 83, 84, 95-98, 103, and 106.

1.2.2 D2 discloses an expression profile carried out on lung tumour samples compared to paired normal lung tissues including determination of expression levels of miR-451 together with miR-486, miR-126, and miR-21. The assay is performed with a microarray chip containing probes for at least these 4 miRNAs.

Therefore, claims 1, 56, 64-66, 68, 69, and 72 are not novel.

1.2.3 D3 discloses the application of miR-451 in preparing a medicine for treating non-small cell lung cancer. Since the recombinant of Pre-miR-451 has influence on propagation, apoptosis, migration and drug resistance of non-small cell lung cancer cells, it is proved that the miR-451 prohibits the propagation, migration and drug resistance of the non-small cell lung cancer cells and promotes the apoptosis of the non-small cell lung cancer cells; and living body level also indicates that the miR-451 has excellent anti-cancer effect (abstract). The document in particular discloses the down-regulation of miR-451 and pre-miR-451 in lung cancer and lung cancer cell lines (Fig. 3 and 4).

Therefore, claims 103 and 106 are not novel.

1.2.4 D4 discloses a complete human miRNA microarray for expression profile studies that doubtlessly includes probes for all the miRNAs disclosed in the present application.

Therefore, claims 65-70, 72-76 and 78-80 are not novel.
1.2.5 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1, 3, 7, 10, 14, 56, 64-70, 72-76, 78-80, 83, 84, 95-98, 103, and 106 is not new in respect of prior art as defined in the regulations (Rule 64(1) - (3) PCT).

1.3 INVENTIVE STEP (Art. 33(3) PCT)

1.3.1 Document D1 is considered to represent the most relevant state of the art. The subject-matter of claim 2 differs in that the level of expression of at least six of the miRNAs is determined. No technical effect can be derived from this difference.

The problem to be solved by the subject matter of claim 2 may therefore be regarded as providing an alternative set of miRNAs. The solution would be at least six of the ones from the tables.

This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons: The miRNAs disclosed in the application are the inevitable result of routine screening based on microarray expression analysis. The skilled person at the date of filing would expect to find differential gene expression when comparing two different samples. Further detailed analysis described in the application is only relative to specific subsets of miRNAs and not to any six of the tables as claimed and it therefore cannot be taken into account for the assessment of the present inventive step.

Moreover, the method as claimed would not confer protection for any specific diagnostic purpose. There is therefore no contribution to the state of the art if it is not known what the different marker expression identifies.

Therefore, claim 2 is not inventive.

1.3.2 Dependent claims 4, 6, 8, 9, 11, 13, 15, 16, 85-94, and 99-102 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step.
1.3.3 Claim 18 further differs in that the method comprises calculating a plurality of real quotients by determining the ratio between the level of expression of at least one pair of miRNA and comparing this ratio with a control value. The technical effect of this difference is the provision of a better diagnostic signature.

This solution cannot be considered inventive for the following reason: The use of ratios between the expression levels of up-regulated versus down-regulated miRNAs is already known in the prior art, for instance from D7, which discloses the ratio of miR-21 to miR-221 from the serum as a robust predictor of NSCLC status compared to healthy subjects. D10 and D11 disclose further examples of miRNA ratios used as markers for cancer diagnosis. The skilled person would therefore be well aware of the possibility to use expression ratios in order to obtain a more predictive signature.

In view of this, claim 18 is not inventive.

1.3.4 For the same reason, claims 19-21, 23-27, and 29-46 are also not inventive. It is important to point out that the specific expression ratios referred to in the subject-matter of these claims are only speculative. As mentioned below, the application does not actually show that all the marker combinations claimed are indeed able to discriminate the tested sample with respect to a control. The proposed solutions in each claim would not therefore represent valid solutions for which it is proven that they are able to solve the problem posed.

1.3.5 Additional dependent claims 47-63 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, involve an inventive step. In particular, claims 57-60 are not inventive because the prior art D8 also discloses a method of determining whether a subject has, or is at risk for developing, one or more lung cancer associated diseases, comprising: measuring the level of at least one miR gene product in a peripheral blood sample from the subject (cl. 1), and wherein the one or more lung cancer associated diseases comprise bronchoalveolar carcinoma (BAC), non-small cell lung cancer (NSCLC), lung adenocarcinoma, and a lung squamous cell carcinoma (cl. 3). D8 further discloses that the study is
conducted on current and former smokers without a history or current diagnosis of lung cancer (previous chest radiograph) along with evaluating a group of healthy never smokers (par. [0103]).

1.3.6 D6 discloses an apparatus able to receive the microarray of D4. Claim 81 differs in that it further comprises means for calculating real quotients. Any computing device would be able to elaborate the expression data and calculate the desired parameters according to the user's set-up. Assembling a microarray reader with a computer is common practice and cannot be considered inventive.

Therefore, claims 81 and 82 are not inventive.

1.3.7 Claims 104-109, 111-115, 117, and 118 differ from D1 in that they refer to various combinations of compounds characterized by miRNAs or inhibitor of miRNAs. As mentioned below, the application does not actually show that all the compounds claimed indeed have some anti-cancer effect. The proposed solutions in each claim would not therefore represent valid solutions for which it is proven that they are able to solve the problem posed. These compounds are only claimed based on mere speculation deriving from expression profiling data.

Therefore, claims 104-109, 111-115, 117, and 118 are not inventive.

1.3.8 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-4, 6-11, 13, 16-21, 23-27, 29-70, 72-76, 80-109, 111-115, 117, and 118 does not involve an inventive step (Rule 65(1)(2) PCT).
Invention 3 (miR-660)
(claims 1-8, 10-15, 17-24, 26-30, 32-73, 75-79, 81-112, 114-118, 120 all partially)

1.4 NOVELTY (Art. 33(2) PCT)

1.4.1 D12 discloses a method of assessing skin cancer in an individual comprising; determining the expression of one or more of the miRNAs selected from a group comprising miR-660, miR-15b and miR-221 (cl. 18); a method of treatment of a skin cancer in an individual comprising increasing the expression or activity of one or more miRNAs selected from a group comprising miR-660 (cl. 22). miR-660 was identified by genome-wide analysis of miRNA expression performed in healthy skin and SCC using the Early Access TaqMan(R) Human MicroRNA Array v1.0 (p. 40, line 11-14).
Therefore, claims 1, 3, 6, 65, 66, 68, 69, 72, 83, and 84 are not novel.

1.4.2 Similarly as above (1.2.4), in view of D4 claims 65-80 are not novel.

1.4.3 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1, 3, 6, 65-80, 83, and 84 is not new in respect of prior art as defined in the regulations (Rule 64(1) - (3) PCT).

1.5 INVENTIVE STEP (Art. 33(3) PCT)

1.5.1 For the same reasoning as in 1.3.1, claim 2 is not inventive.

1.5.2 Independent claims 7 and 14 differ from D12 in that the method identifies an aggressive tumour. Independent claim 10 differs in that miR-660 is detected with at least a miRNA from Tables Vb and Vd.

As already said above (1.3.1), the miRNAs disclosed in the application are the inevitable result of routine screening based on microarray expression analysis. The skilled person at the date of filing would expect to find differential gene expression when comparing two different samples, comprising samples for
any type of tumour. Furthermore, the detailed association analysis described in the application is only specific to subsets of miRNAs and not to any combination of miRNAs as claimed and it therefore cannot be taken into account for the assessment of the present inventive step for these claims.

Therefore, claims 7, 10 and 14 are not inventive.

1.5.3 Dependent claims 4, 5, 8, 11-13, 15, 17, 60-64, 67, 70, 71, 73, 75-79 and 85-102 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step.

1.5.4 Claim 56 differs in that the tumour is pulmonary tumour. Again, the claim is not inventive because, in view of the fact that only specific combinations of miRNAs are indeed shown to be useful for the identification of pulmonary tumour, the problem is considered not solved over the whole breadth of the proposed solution. Similarly, claims 57-59 are also not inventive.

1.5.5 The same reasoning as at points 1.3.3-1.3.6 can be applied to this invention. Therefore, dependent claims 18-24, 26-30, 32-55, 58, 59 81 and 82 are not inventive.

1.5.6 D12 further discloses a method of screening for a compound useful in the treatment of skin cancer comprising; contacting a cell with a test compound and; determining the expression of one or more miRNAs, comprising miR-660 (cl. 39, 48), further comprising formulating said test compound in a pharmaceutical composition with a pharmaceutically acceptable excipient, vehicle or carrier (cl. 53). Claim 103 differs in that the compound comprises miR-660 itself or an inhibitor thereof. This solution is obvious to the skilled person in view of D13, which discloses that pharmaceutical compositions may comprise either the miRNA as such if it needs to be overexpressed or an inhibitor of it if it needs to be repressed according to its expression in tumour.

Therefore, claim 103 is not inventive.
1.5.7 Accordingly and for the same reason as at point 1.3.7, claims 104-112, 114-118, and 120 are also not inventive.

1.5.8 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-8, 10-15, 17-24, 26-30, 32-73, 75-79, 81-112, 114-118, and 120 does not involve an inventive step (Rule 65(1)(2) PCT).

Invention 9
(claims 1-120 all partially)

1.6 NOVELTY (Art. 33(2) PCT)

1.6.1 D8 discloses a miRNA chip used to idenfiy miRNAs isolated from whole blood, PBMC, serum and lung tissue. The microarray facility utilized a microRN ACHIP v3 that contains probes against 578 precursor miRNA sequences (par. [000112]). Using this microarray, miR-197 is identified among miRNAs that are increased and decreased in lung cancer relative to normal levels in serum (ex. 7, Table 3). The microarray comprises therefore probes specific for miR-197. Moreover, miR-320 is also identified (Table 2).

Therefore, claims 65, 66, 68, and 69 are not novel.

1.6.2 Similarly as above (1.2.4), in view of D4 claims 65-80 are not novel.

1.6.3 D13 suggests a pharmaceutical composition for treating lung cancer, comprising at least one miR expression-inhibitor compound and a pharmaceutically-acceptable carrier (cl. 29), wherein the at least one miR expression-inhibitor compound is specific for a miR gene product selected from a group comprising miR-197 (cl. 31). The document therefore anticipates the subject-matter of claims 103 and 106.
1.6.4 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 65-80, 103 and 106 is not new in respect of prior art as defined in the regulations (Rule 64(1) - (3) PCT).

1.7 INVENTIVE STEP (Art. 33(3) PCT)

1.7.1 Document D8 is considered to represent the most relevant state of the art and discloses a method of determining whether a subject has, or is at risk for developing, one or more lung cancer associated diseases, comprising measuring the level of at least one miR gene product in a peripheral blood sample from the subject and comparing an alteration in the level of the miR gene product in the sample relative to the level of a corresponding miR gene product in a control sample (cl.1), wherein the sample comprises serum, and wherein at least one miR gene product is one or more miR gene products selected from the group of hsa-miR- 532, hsa-miR-197, hsa-miR-342 (cl. 10) and wherein the one or more lung cancer associated diseases comprise bronchoalveolar carcinoma (BAC), non-small cell lung cancer (NSCLC), lung adenocarcinoma, and a lung squamous cell carcinoma (cl. 3). D8 further discloses that the study is conducted on current and former smokers without a history or current diagnosis of lung cancer (previous chest radiograph) along with evaluating a group of healthy never smokers (par. [0103]). The subject-matter of claim 1 differs in that miR-197 is determined together with one of the other miRNAs listed in Tables Ia, Ic, IIa, IIc, Va, Vc, Vla or Vlc. No technical effect can be derived from this difference.

The problem to be solved by the subject matter of claim 1 may therefore be regarded as providing an alternative set of miRNAs to determine together with miR-197. The solution would be one of the other miRNAs listed in Tables Ia, Ic, IIa, IIc, Va, Vc, Vla or Vlc.

This solution cannot however be considered as involving an inventive step (Article 33(3) PCT) for the following reasons: The miRNAs disclosed in the application are the inevitable result of routine screening based on microarray expression analysis. The skilled person at the date of filing would expect to find differential gene expression when comparing two different samples. Further detailed analysis described in the application is only relative to specific
subsets of miRNAs and not to any combination from the tables as claimed and it therefore cannot be taken into account for the assessment of the present inventive step.

Moreover, the method as claimed would not confer protection for any specific diagnostic purpose. There is therefore no contribution to the state of the art if it is not known what the different marker expression identifies.

Therefore, claim 1 is not inventive.

1.7.2 For the same reason, independent claims 3, 7, 10, and 14, are also not inventive.

1.7.3 Dependent claims 2, 4-6, 8, 9, 11-13, 15-17, 58-64, 83-102 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT in respect of inventive step. In particular, claims 5, 8, 9, 11, 12, 15, and 16 make reference to specific miRNA combinations. These combinations are however merely provided as preferred embodiments without actually showing their diagnostic relevance. For this reason, they are considered as arbitrary selections of miRNAs.

1.7.4 For the same reasons explained at points 1.3.3 and 1.3.4, claims 18-57 are also not inventive.

1.7.5 For the same reasons explained at point 1.3.6, claims 81 and 82 are also not inventive.

1.7.6 In view of D13 and of the same reason as at point 1.3.7, claims 104-120 are not inventive.

1.7.7 The present application does therefore not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-120 does not involve an inventive step (Rule 65(1)(2) PCT).
1.8 WARNING ABOUT ENTRY IN THE EUROPEAN PHASE

Claims 83-102 refer to methods comprising treatment steps. The search has been carried out and based on the alleged effects. Patenting in the European phase will however depend on the formulation of the claims, because the EPO does not patent applications claiming medical treatments.

VI. **Certain documents cited** (Continuation)

D14 was published on 01.03.2011. Although it does not constitute prior art within the meaning of Rule 64.1(b), it appears to disclose all the features of the claims. It seems that at the moment the claimed subject-matter is comprised in the disclosure of the priority documents dated 07.02.2011. Should this not to be the case at later checks, the document may be taken into account to reassess the patentability of the application.

VIII. **Certain Observations** (Continuation)

1 Present claims 1-120 relate to an extremely large number of possible compounds/products/apparatus/methods. Support and disclosure in the sense of Articles 6 and 5 PCT is to be found however for only a very small proportion of the compounds/products/apparatus/methods claimed.

The present application provide supporting data only for the marker ratio signatures described in Figures 4A-4D, the effect of transfection with miR-486 and miR-660 (ex. 2) and the expression profiles of Tables XII and XIII.

The data shown in Figures 5-8 about the expression ratios of Tables III, IV, VII and VIII are not sufficient because no information about the sample size and the statistical significance is provided.

In view of this, claims 1-120 do not satisfy the criterion of Articles 6 and 5 PCT.
Claims 3, 7, 10, and 14 refer to methods for determining the presence of any tumour. The data in the application are however derived from the analysis of pulmonary tumour. Support and disclosure are therefore limited to this type of tumour.

Claims 16 and 80 refer to miRNA lists in Tables Vle and Vlf comprising miR-451 but they are dependent on claims 14 and 78 respectively that refer to Tables Vlb and Vld which do not comprise miR-451. This creates an inconsistency in the subject-matter of these claims.