PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter I of the Patent Cooperation Treaty)
(PCT Rule 44bis)

<table>
<thead>
<tr>
<th>Applicant’s or agent’s file reference</th>
<th>FOR FURTHER ACTION</th>
<th>See item 4 below</th>
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<tr>
<th>International application No.</th>
<th>International filing date (day/month/year)</th>
<th>Priority date (day/month/year)</th>
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International Patent Classification (8th edition unless older edition indicated)
See relevant information in Form PCT/ISA/237

Applicant
NANOBAC PHARMACEUTICALS INCORPORATED

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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 44bis.1(a).

2. This REPORT consists of a total of 12 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:

- Box No. I  Basis of the report
- 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 Article 35(2) with regard to novelty, inventive step or 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

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
11 June 2008 (11.06.2008)

Authorized officer
Philippe Becamel

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1211 Geneva 20, Switzerland

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

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

PCT

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

Date of mailing
(day/month/year) see form PCT/ISA/210 (second sheet)

FOR FURTHER ACTION
See paragraph 2 below

Applicant's or agent's file reference
see form PCT/ISA/220

International application No.
PCT/US2005/044589

International filing date (day/month/year)
09.12.2005

Priority date (day/month/year)

International Patent Classification (IPC) or both national classification and IPC
INV. G01N33/569 C12Q1:04

Applicant
NANOBAC LIFE SCIENCES, INC.

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 or 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/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office - P.B. 5818 Patentenweg - NL-2280 HV Rijswijk - Pays Bas
Tel. +31 70 340 - 2040 Tx: 31 651 epo nl
Fax: +31 70 340 - 3016

Date of completion of this opinion
See form PCT/ISA/210

Authorized Officer
Van Bohemen, Charles
Telephone No. +31 70 340-2199

Form (PCT/ISA/237) (Cover Sheet) (April 2005)
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. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:
   a. type of material:
      - [ ] a sequence listing
      - [ ] table(s) related to the sequence listing
   b. format of material:
      - [ ] on paper
      - [ ] in electronic form
   c. time of filing/furnishing:
      - [ ] contained in the international application as filed.
      - [ ] filed together with the international application in electronic form.
      - [ ] furnished subsequently to this Authority for the purposes of search.

3. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto 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.

4. Additional comments:
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

<table>
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<tr>
<th>Category</th>
<th>Yes: Claims</th>
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<td>Novelty (N)</td>
<td>2, 28, 45, 51, 60, 67-79, 100-104, 107</td>
<td>1, 3-27, 29-44, 46-50, 52-59, 61-65, 80-99, 105-106</td>
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<td>Inventive step (IS)</td>
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<td>Industrial applicability (IA)</td>
<td>1-107</td>
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2. Citations and explanations

see separate sheet

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
Re Item I
Basis of the report

The application as filed.

Re Item V
Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

Reference is made to the following document/s; the numbering will be adhered to in the rest of the procedure:


Analysis

The present application (PA) discloses methods and compositions for detecting, analysing and assessing the significance of calcifying nano-particles (CNPs), also known as nanobacteria (cf. D3, page 1, paragraph 3). CNPs are approximately 200 microns in size and appear to multiply in the biological mode, meaning their growth curve has the same characteristics as that of a life form, i.e., certain doubling time (typically around 3 days), plus a lag, a logarithmic, a stationary and even a death phase. The particles are passageable apparently indefinitely in cell culture media. The main structural component identified is bone-like. CNPs have been clearly differentiated from known biological entities: eubacteria, archaea-bacteria, virus, prions and eukaryotes. CNPs have been shown to form mineral calcium or hydroxy apatite coatings on their surfaces. The hydroxy apatite
surface acts as a mineral calcium substrate for the binding of calcium binding proteins. Proteins that associate with the CNP Hydroxyapatite may undergo a conformational change. Subsequently, said complex may attract or bind other proteins that have an affinity to the aforementioned complex. The disclosed methods and compositions generally involve detecting one or more proteins present on a calcifying nano-particle. It has been discovered that particular proteins become associated with calcifying nanoparticles. This association provides a means for detecting, classifying, analysing, categorizing, and assessing calcifying nano-particles. Detecting particular proteins while associated with a calcifying nano-particle can be used to indicate the presence and type of calcifying nano-particle, which can be used to indicate the presence of, or disposition to, diseases or conditions. Multiple proteins on a calcifying particle can be detected. The presence or absence of particular proteins and the pattern of the presence and absence of particular proteins can be used to indicate the presence and type of calcifying nanoparticle.

**Objections**

1. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 1 is not new in the sense of Article 33(2) PCT.

Document D1, which is considered to represent the most relevant state of the art, discloses a method to detect CNPs by detecting proteins (or nucleic acid) on said CNPs using monoclonal antibodies to CNPs. Document D2 discloses an ELISA kit to detect CNPs by detecting proteins on said CNPs. The subject-matter of independent claim 1 does not appear to differs from the above at all (cf. D1, figure 1; D2, pages 1-2).

The subject-matter of dependent claims 2, 28 and 45 in combination with independent claim 1, does not appear to have been disclosed in the prior art. Its incorporation into the present independent claim 1 could perhaps restore novelty and inventivity ex Article 33(1-3) PCT to said independent claim.

Incorporation of the subject-matter disclosed in dependent claims 3-27, 29-44 or 46-49 into the present independent claim 1 does not appear to be able to restore novelty to said independent claim, as it too has been disclosed in the document D1 or closely related prior art or pertains to one or more features which are merely one of several straight-
forward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill or undue experimentation.

2. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 50 is not new in the sense of Article 33(2) PCT according to the same reasoning given above.

The subject-matter of dependent claim 51 in combination with independent claim 50, does not appear to have been disclosed in the prior art. Its incorporation into the present independent claim 1 could perhaps restore novelty and inventivity ex Article 33(1-3) PCT to said independent claim.

Incorporation of the subject-matter disclosed in dependent claims 52-58 into the present independent claim 50 does not appear to be able to restore novelty to said independent claim, as it too has been disclosed in the document D1 or closely related prior art or pertains to one or more features which are merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill or undue experimentation.

3. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 59 is not new in the sense of Article 33(2) PCT according to the same reasoning given above in point 1.

The subject-matter of dependent claims 60 and 100 in combination with independent claim 59, does not appear to have been disclosed in the prior art. Its incorporation into the present independent claim 59 could perhaps restore novelty and inventivity ex Article 33(1-3) PCT to said independent claim.

Incorporation of the subject-matter disclosed in dependent claims 61-66 into the present independent claim 59 does not appear to be able to restore novelty to said independent claim, as it too has been disclosed in the document D1 or closely related prior art or pertains to one or more features which are merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances,
without the exercise of inventive skill or undue experimentation.

4. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 80 is not new in the sense of Article 33(2) PCT according to the same reasoning given above in point 1.

Documents D1 and D2 disclose compositions comprising CNPs and compounds, i.e. monoclonal antibodies, bound to said CNPs (cf. D1 and D2 above point 1). The subject-matter of independent claim 80 does not appear to differ from the above at all.

Incorporation of the subject-matter disclosed in dependent claims 81-84 into the present independent claim 80 does not appear to be able to restore novelty to said independent claim, as it too has been disclosed in the document D1 or closely related prior art or pertains to one or more features which are merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill or undue experimentation.

5. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 85 is not new in the sense of Article 33(2) PCT according to the same reasoning given above in point 4.

Incorporation of the subject-matter disclosed in dependent claims 86-98 into the present independent claim 85 does not appear to be able to restore novelty to said independent claim, as it too has been disclosed in the document D1 or closely related prior art or pertains to one or more features which are merely one of several straightforward possibilities from which the skilled person would select, in accordance with circumstances, without the exercise of inventive skill or undue experimentation.

6. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 99 is not new in the sense of Article 33(2) PCT.

Document D2 discloses a kit (to detect CNPs) comprising detection compounds and solid supports. The subject-matter of independent claim 99 does not appear to differ from the above at all.
For completeness it is noted that the designation "for the detection...thereupon" is not regarded as a characterizing feature of the kit of claim 99. The applicant should have disclosed which technical features can always and unambiguously distinguish the kit of claim 99 from those kits disclosed in the prior art, e.g. D2.

7. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 105 is not new in the sense of Article 33(2) PCT according to the same reasoning given above in point 1.

8. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 106 is not new in the sense of Article 33(2) PCT according to the same reasoning given above in point 1.

9. The PA does not meet the criteria of Article 33(1) PCT, because the subject-matter of independent claim 104 does not appear to involve an inventive step in the sense of Article 33(3) PCT.

The document D1 is regarded as being the closest prior art to the subject-matter of claim 104, and discloses a method to detect CNPs in biological tissue samples by detecting proteins on said CNPs using monoclonal antibodies to CNPs (cf. D1, figure 1).

The subject-matter of claim 104 therefore differs from D1 in that tissue samples have been replaced by "foreign devices implanted or to be implanted". However, this would be merely one of several straightforward possibilities to apply the method of D1 from which the artisan would select, in accordance with circumstances, without the exercise of inventive skill or undue experimentation. Furthermore, the description of the PA does not disclose any unexpected advantages of the method of claim 104 vis à vis the method of D1.

Conclusion.

In consequence, the methods and particles of independent claims 67, 73, 79, 103 and 107 of the PA are considered to be novel, involving an inventive step and industrially applicable (PCT Article 33(1) - (4) PCT). Claims 68-72 and 74-78 are dependent on claims 67 and 73
respectively, and as such also meet the requirements of the PCT with respect to novelty, inventive step and industrial applicability.

As presently worded, the methods and compositions of claims 1-66, 80-99 and 104-106 of the PA are not considered to be novel and involving an inventive step ex Article 33(1) - (3) PCT. However, said claims are considered to be industrially applicable.

Re Item VIII
Certain observations on the international application

1. Clarity ex Article 6 PCT could have been enhanced by noting in claim 1 and in the description on page 1, line 11 that CNPs are also known as nanobacteria (cf. D3, page 1, paragraph 3).

2. The PA does not meet the requirements of Article 6 PCT, because independent claims 1, 59, 67, 73, 80, 85 and 103 - 107 are not clear, because the artisan would not immediately understand what is intended by the term "components on the CNP".

3. The PA does not meet the requirements of Article 6 PCT, because independent claim 79 is not clear, because the artisan would not immediately understand what is intended by the term "isolated CNP"; i.e. the degree of "isolation" or "purity" would not be automatically clear.

4. Although claims 79, i.e. an isolated CNP, and 80, i.e. a composition comprising a CNP with a compound bound to it, have been drafted as separate independent claims, they appear to relate effectively to the same subject-matter and to differ from each other only with regard to the definition of the subject-matter for which protection is sought. The aforementioned claims therefore lack conciseness and as such do not meet the requirements of Article 6 PCT.

5. It is clear from the description on page 1, first paragraph that the following feature is essential to the definition of the invention: the particles of the PA are calcifying nanoparticles.
Since independent claim 85 does not contain this feature it does not meet the requirement following from Article 6 PCT taken in combination with Rule 6.3(b) PCT that any independent claim must contain all the technical features essential to the definition of the invention.

6. The PA does not meet the requirements of Article 6 PCT, because dependent claims 100-102 are not clear, because the artisan would not immediately understand what is intended by the phrase "said pattern of said proteins", because the independent claims to which claims 100-102 refer back to (i.e. claims 59, 67 and 73) do not disclose a pattern of proteins.

7. The PA does not meet the requirements of Article 6 PCT, because claim 67 is not clear, because the artisan would not immediately understand how the method of claim 67 could be workable; i.e. how the identification of proteins on CNPs results in the prognosis of a disease.

8. The PA does not meet the requirements of Article 6 PCT, because claim 103 is not clear, because the artisan would not immediately understand how the method of claim 103 could be workable; i.e. how the identification of proteins on CNPs results in the identification of a treatment.

9. The PA does not meet the requirements of Article 6 PCT, because claim 107 is not clear, because the artisan would not immediately understand how the method of claim 107 could be workable; i.e. how the detection of proteins on CNPs results in the determination of risk of future severe adverse health.

10. The PA does not meet the requirements of Article 6 PCT, because claim 107 is not clear, because the artisan would not immediately understand what is intended by the term "severe adverse health"; i.e. said term appears to be undefined and subjective.

11. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D1-D3 is not mentioned in the description, nor are these documents identified therein.
12. The application does not meet the requirements of Article 6 PCT, because the description of the PA is not clear, because the artisan would not immediately understand what is intended by the phrase on page 2, second paragraph of the description, "assigned patents and patent applications incorporated by reference"; i.e. the technical features intended would not automatically be clear to the artisan. Also, said phrase implies that the subject-matter for which protection is sought may be different to that defined by the claims, thereby resulting in lack of clarity when used to interpret them.

13. Clarity ex Article 6 PCT could have been enhanced by replacing the patent application numbers on page 2, line 4 of the description by published patent numbers or published patent application numbers.