PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

India49 NATTAR MAIN STREET
MURUNGAPAKKAM MUDALIARPET
PUDUCHERRY-605 004 INDIA

DR.LAKSHMANANE BOOMINATHAN

PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43 *bis*.1)

Date of mailing *(day/month/year)*	15 Mar. 2012 (15.03.2012)
Applicant's or agent's file reference	**FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/IN2011/000684	30 Sep. 2011(30.09.2011)	30 Sep. 2010(30.09.2010)

International Patent Classification (IPC) or both national classification and IPC

See Supplemental Box **II**

Applicant

DR. LAKSHMANANE BOOMINATHAN

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 43*bis*.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 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 66.1*bis*(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/CN	Date of completion of this opinion	Authorized officer
The State Intellectual Property Office, the P.R.China 6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China 100088 Facsimile No. 86-10-62019451	07 Mar. 2012 (07.03.2012)	XING,Weiling Telephone No. (86-10)62414331

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IN2011/000684

Box No. I 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. a sequence listing filed or furnished
      ☐ on paper
      ☐ in electronic form

   b. time of filing or furnishing
      ☐ contained in the applicant as filed
      ☐ filed together with the application in electronic form
      ☐ furnished 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:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IN2011/000684

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

1. Statement:

Novelty (N)	Claims	2-10	YES
	Claims	1	NO
Inventive step (IS)	Claims	None	YES
	Claims	1-10	NO
Industrial applicability (IA)	Claims	1-10	YES
	Claims	None	NO

2. Citations and explanations

Reference is made to the following documents:

D1: SACHDEVA, M. et al. p53 represses c-Myc through inductionof the tumor suppressor miR-145.PNAS. 3 March 2009 (03.03.2009), vol. 106, No. 9, pages 3207–3212.

D2: SHU, Limin et al. RNPC1, an RNA-binding protein and a target of the p53 family, is required for maintaining the stability of the basal and stress-induced p21 transcript. Genes Dev. 18 October 2006(18.20.2006), vol.20, pages 2961-2972.

D3: LEE,Y.S. et al. The tumor suppressor microRNA let-7 represses the HMGA2 oncogene, Genes Dev. 16 April 2007 (16.04.2007), vol. 21, pages 1025-1030.

D4: FAN,C. et al. PTEN inhibits BMI1 function independently of its phosphatase activity.Molecular Cancer.10 November 2009 (10.11.2009), vol. 8, pages 98-111.

The subject-matter of claim 6 is the stable cell line as stated in 5, however,claim 5 does not appear in the expression of stable cell line, thereby rendering the definition of claims 6 unclear. The opinion has been carried out and based on claim 6 :The stable cell line, generated by the expression vector as stated in 5,will be used to screen for compounds that simultaneously suppress RNPC1 and induce tumor suppressor p63……futher evaluation.

I. Novelty

1.D1 is regarded as the closest prior art to claim 1 and discloses: cells that expressed both c-myc and tumor suppressor miR-145 promoters.To determine whether miR-145 directly targets c-Myc, constructed a luciferase reporter carrying c-Myc 3-UTR with a putative miR-145 binding site. We detected a reduction of luciferase activity by50% in the miR-145 transfected cells as compared with vector control. (see page 3209 right column paragrph 4, Figtures 4E and 4F).Therefore,the technical solution about "A stable cell line that expresses both c-myc and tumor suppressor miR-145" of claim 1 is disclosed by document D1, and the subject-matter of claim 1 is not new in the sense of Article 33(2) PCT. Because D1 does not disclose the cell line that expresses both c-myc and other tumor suppressor in addition to miR-145, therefore, the corresponding technical solution of claim 1 is new in the sense of Article 33(2) PCT.

2. D1 does not disclose miR-145 promoter will be linked to firefly luciferase, and firefly luciferase is linked to miR-145 promoter. Hence, claim 2 is new in the sense of Article 33(2) PCT.

3.D1 does not disclose the expression vector which be used to generate stable cell line that expresses both c-myc and miR-145. Hence, claim 3 is new in the sense of Article 33(2) PCT.

4. D1 does not disclose the stable cell lines which is generated by expression vector as stated in claim 3 Hence, claim 4 is new in the sense of Article 33(2) PCT.

5. D2 is regarded as the closest prior art to claim 5 and claim 6 and discloses: a dual luciferase assay is performed in triplicate according to the manufacturer's instructions (Promega). Briefly, p53-null H1299 cells are plated at 5 × 104 cells per well in a 24-well plate and allowed to recover overnight. Cells are then cotransfected with 200 ng each of pGL2, O-Fluc, or pGL3 together with pcDNA3 or a pcDNA3 vector expressing wild-type p53, mutant p53(R249S), p63, p73, or RNPC1a. As an internal control, 5 ng of pRL-CMV, a Renilla luciferase vector (Promega), is also cotransfected per well（see D2 page 2970 left column paragrph 2）.The difference between the subject-matter of claim 5 and the disclosure of D2 is that RNPC1 and p53 are cloned into a single mammalian expression vector. Hence, claim 5 and dependent claim 6 are new in the sense of Article 33(2) PCT.

6.D3 is regarded as the closest prior art to claim 7 and claim 8 and discloses: let-7 is introduced into H1299 cells by transfecting a short RNA duplex containing let-7 sequence on one strand. Luciferase reporters containing artificial let-7 target sites in the 31UTR are repressed by such duplexes transfection of let-7 duplex to H1299 reduced the HMGA2 mRNA and protein (see D3 page 1027 left column paragrph 3,Figs. 2A,2B). The difference between the subject-matter of claim 7 and the disclosure of D3 is that claim 7 is let-7 is linked to renilla luciferase. Hence, claim 7 and dependent claim 8 are new in the sense of Article 33(2) PCT.

(see Supplemental Box I)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/IN2011/000684

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:

The subject-matter of claim 6 is the stable cell line as stated in 5, however,claim 5 does not appear in the expression of stable cell line, thereby rendering the definition of claims 6 unclear, contrary to the requirements of Article 6 PCT. The search has been carried out and based on claim 6 :The stable cell line, generated by the expression vector as stated in 5,will be used to screen for compounds that simultaneously suppress RNPC1 and induce tumor suppressor p63……futher evaluation.

Supplemental Box I

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of : box V: 2. Citations and explanations

7.D1-D3 all do not disclose components of the biological pathways mentioned in claim 9.Hence, claim 9 is new in the sense of Article 33(2) PCT.

8. D4 is regarded as the closest prior art to claim 10 and discloses: a retrovirus expressing BMI1, PTEN, or both BMI1 and PTEN are infected into DU145 cells (see D4 page 9 of 14, left column paragrph 3,Fig 6). Nuclear PTEN suppresses BMI1 function. PTEN binds to BMI1 in the nucleus of prostate cancer cells and reduces BMI1-mediated suppression of p16INK4A and p14ARF as well as BMI1-mediated enhancement of hTERT. Additionally, PTEN co-localizes with BMI1 more frequently in primary prostate carcinomas compared to normal prostate glands(see D4 page 2 of 14,right column paragrph 2). The difference between the subject-matter of claim 10 and the disclosure of D4 is that BMI1 is linked to renilla luciferase and PTEN is linked to firefly luciferase.Hence, claim 10 is new in the sense of Article 33(2) PCT.

II. Inventiveness:

1. D1 discloses p53 represses c-Myc through induction of the tumor suppressor miR-145. To determine whether miR-145 directly targets c-Myc, a luciferase reporter carrying c-Myc 3 -UTR with a putative miR-145 binding site is constructed.To determine whether p53 activates the miR-145 promoter, a 1.4-kb putative miR-145 promoter carrying the 2 p53 response elements (p53REs) is cloned into a pGL3 basic vector to generate pMir-145p-Luc-1. Tested pMir-145p-Luc-1 in NIH/3T3 cells. p53 increased miR-145 promoter activity by 7-fold. This p53 induction of luciferase activity is specific to the miR-145 promoter. They also tested pMir-145p-Luc-1 in MCF-7 cells. Similarly, p53 also increased miR-145 promoter activity in MCF-7 cells (see page 3208 right column paragrph 1, Figs. 3A and 3B).These experiments show that the tumor suppressor miR-145 represses c-Myc, and p53 represses c-Myc through induction of the tumor suppressor miR-145. Although D1 does not disclose the combination of c-myc promoter plus other tumor suppressor ,for example TAP63/p73/INK4a/CDKN1a /ARF and so on, it is known in the art that TAP63/p73/INK4a/CDKN1a /ARF and miR-145 are the same as tumor suppressor.In contrast to the D1 discloses miR-145 and c-myc combinantion, the person skilled in the art can conduct a genetic screen to identify compounds that suppress the expression of c-myc and induce the expression of tumor suppressor miRNAs/genes. Hence, claim 1 does not involve an inventive step in the sense of Article 33(3) PCT.

2. D1 discloses cells that expressed both c-myc and tumor suppressor miR-145 promoter(see page 3209 right column paragrph 4, Figtures 4E and 4F). Although D1 does not disclose luciferase linked to c-myc is renilla luciferase，and firefly luciferase is linked to miR-145 promoter, It is known in the art that renilla luciferase or firefly luciferase is used to detect gene expression, so in the light of D1, it is obvious to the person skilled in the art to link renilla luciferase with c-myc, and link firefly luciferase with miR-145. In addition,using expression vector containing resistance marker to transfect cell is well known in the art. Hence, claim 2 does not involve an inventive step in the sense of Article 33(3) PCT.

3. D1 discloses: to determine whether p53 activates the miR-145 promoter, a 1.4-kb putative miR-145 promoter carrying the 2 p53 response elements (p53REs) is cloned into a pGL3 basic vector to generate pMir-145p-Luc-1. pMir-145p-Luc-1 in NIH/3T3 cells and MCF-7 cells is tested. P53 increased miR-145 promoter activity in NIH/3T3 cells and MCF-7 cells (see page 3208 right column paragrph 1, Figures 3A and 3B).This shows expression vector is used to generate stable cell clones. The person skilled in the art will be obvious to construct the expressing vector which generated cell lines and the described cell line. Hence, claim 3 and claim 4 does not involve an inventive step in the sense of Article 33(3) PCT.

Additionaly, D1 discloses:to determine whether miR-145 directly targets c-Myc, a luciferase reporter carrying c-Myc 3 -UTR with a putative miR-145 binding site is constructed. To determine whether p53 activates the miR-145 promoter, a 1.4-kb putative miR-145 promoter carrying the 2 p53 response elements (p53REs) is cloned into a pGL3 basic vector to generate pMir-145p-Luc-1. tested pMir-145p-Luc-1 in NIH/3T3 cells. p53 increased miR-145 promoter activity by 7-fold. This p53 induction of luciferase activity is specific to the miR-145 promoter. They also tested pMir-145p-Luc-1 in MCF-7 cells. Similarly, p53 also increased miR-145 promoter activity in MCF-7 cells (see page 3208 right column paragrph 1, Fig. 3A and 3B).These experiments show that the tumor suppressor miR-145 represses c-Myc, and p53 represses c-Myc through induction of the tumor suppressor miR-145. Although D1 does not disclose other combination of c-myc promoter plus other tumor suppressor ,for example TAP63/p73/INK4a/CDKN1a /ARF and so on, It is known in the art that TAP63/p73/INK4a/CDKN1a /ARF and miR-145 are the same as the tumor suppressor.In contrast to the D1 discloses miR-145 and c-myc combinantion, the person skilled in the art can conduct a genetic screen to identify compounds that suppress the expression of c-myc and induce the expression of tumor suppressor miRNAs/genes. Hence, claim 4 does not involve an inventive step in the sense of Article 33(3) PCT.

5. D2 is regarded as the closest prior art to claim 5 and claim 6 and discloses: a dual luciferase assay is performed in triplicate according to the manufacturer's instructions (Promega). Briefly, p53-null H1299 cells are plated at 5 × 104 cells per well in a 24-well plate and allowed to recover overnight. Cells are then cotransfected with 200 ng each of pGL2, O-Fluc, or pGL3 together with pcDNA3 or a pcDNA3 vector expressing wild-type p53, mutant p53(R249S), p63, p73, or RNPC1a. As an internal control, 5 ng of pRL-CMV, a Renilla luciferase vector (Promega), is also cotransfected per well（see D2 page 2970 left column paragrph 2）. The difference between the subject-matter of claim 5 and the disclosure of D2 is that RNPC1 and p53 are cloned into a single

(see Supplemental Box II)

Supplemental Box II

In case **the space in any of the preceding boxes is not sufficient.**

Continuation of : Supplemental Box I

mammalian expression vector. However, D2 discloses RNPC1 is a direct target of the p53 family(see page 2962 the left column), it is obvious to the person skilled in the art to clone RNPC1 and p53 into a single mammalian expression vector and transfect the vector into cell line. Hence, claim 5 and claim 6 do not involve an inventive step in the sense of Article 33(3) PCT.

Although D2 does not disclose other combination of RNPC1 promoter plus other tumor suppressor ,for example miR-14/16/let-7 and so on, It is known in the art that miR-14/16/let-7 and p53 are the same as the tumor suppressor.In contrast to the D2 discloses RNPC1 and p53 combinantion, the person skilled in the art can conduct a genetic screen to identify compounds that suppress RNPC1 and induce the expression of tumor suppressor miRNAs/genes. Hence, claims 5 and 6 do not involve an inventive step in the sense of Article 33(3) PCT.

6 D3 is regarded as the closest prior art to claim 7 and claim 8 and discloses: let-7 is introduced into H1299 cells by transfecting a short RNA duplex containing let-7 sequence on one strand. Luciferase reporters containing artificial let-7 target sites in the 31UTR are repressed by such duplexes transfection of let-7 duplex to H1299 reduced the HMGA2 mRNA and protein (see D3 page 1027 left column paragrph 3,Figs. 2A,2B). The difference between the subject-matter of claim 7 and the disclosure of D3 is that let-7 is linked to renilla luciferase. It is known in the art that renilla luciferase is a very conventional luciferase. Hence, claim 7 does not involve an inventive step in the sense of Article 33(3) PCT.

Although D3 does not disclose the mammalian cell line that expresses Dicer1/Pax5/CDC6/ARK1/2/MYCN. It is known in the art that Dicer1/Pax5/CDC6/ARK1/2/MYCN and let-7 are the same as the tumor suppressor.In contrast to the D3, the person skilled in the art can conduct a mammalian cell line that expresses Dicer1/Pax5/CDC6/ARK1/2/MYCN. Hence, claim 7 does not involve an inventive step in the sense of Article 33(3) PCT.

It is obvious to the person skilled in the art to use as described cell line to screen for compounds that induce or suppress let-7. Hence, claim 8 does not involve an inventive step in the sense of Article 33(3) PCT.

7. D1 discloses: p53 represses c-Myc through induction of the tumor suppressor miR-145. The tumor suppressor p53 is a master gene regulator controlling diverse cellular pathways, by either activating or repressing downstream genes. Among such genes is the proto-oncogene c-Myc, which is negatively regulated by p53. As a transcription factor, c-Myc regulates numerous genes directly or indirectly and thus plays an important role in cellular processes such as development,differentiation, cell proliferation and apoptosis. Deregulated expression of c-Myc has been detected in a wide variety of human cancers(see D1 page 3207 left column paragrph 1).It is obvious to the penson skilled in the art to use the component of p53/ P53 homologue and c-myc/ c-myc homologue and miR-145/miR-145 homologue for diagnosis or treatment of disease,includig cancer. Hence, claim 9 does not involve an inventive step in the sense of Article 33(3) PCT.

8. The difference between the subject-matter of claim 10 and the disclosure of D4 is that BMI1 is linked to renilla luciferase and PTEN is linked to firefly luciferase. It is known in the art that renilla or firefly luciferase is a very conventional luciferase. Hence, claim 10 does not involve an inventive step in the sense of Article 33(3) PCT.

III. Industrial applicability:

The inventions of claims 1-10 can find industrial applicability in the technical field of cancer therapy technology, and thus meet the requirement of Article 33(4) PCT.

Continuation of: International Patent Classification (IPC) or both national classification and IPC of Cover Sheet

C12N5/00 (2006.01)i

C12N 5/09 (2010.01)i

C12N15/79 (2006.01)i

A61K48/00 (2006.01)i