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C12Q 1/42 (2006.01)  GOIN 33/574 (2006.01)

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(71) Applicant (for all designated States except US): HISTORX, INC., [US/US]; 300 George Street, New Haven, CT 06511 (US).

(72) Inventors; and


(54) Title: CORRELATION OF MOLECULAR MARKERS WITH CLINICAL OUTCOME IN GBM PATIENTS RADIATION TREATED WITH OR WITHOUT GEFITINIB

Figure 1.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Antibody Impurity</th>
<th>Number</th>
<th>Catalog #</th>
<th>Localisation</th>
<th>Year</th>
<th>Dose (mg/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pAKT</td>
<td>Mouse</td>
<td>CST</td>
<td>#604</td>
<td>Radian</td>
<td>1.25</td>
<td>2 µg/ml</td>
</tr>
<tr>
<td>pEGFR</td>
<td>Rabbit</td>
<td>CST</td>
<td>#513</td>
<td>Dose and nuclear</td>
<td>1.25</td>
<td>2.5 µg/ml</td>
</tr>
<tr>
<td>EGFR vki</td>
<td>Rabbit</td>
<td>Affinity Antibody</td>
<td>PDB-00820</td>
<td>Complementary</td>
<td>1.20</td>
<td>3 µg/ml</td>
</tr>
<tr>
<td>pTkR</td>
<td>Mouse</td>
<td>#610</td>
<td>#444</td>
<td>Complementary</td>
<td>1.25</td>
<td>3 µg/ml</td>
</tr>
<tr>
<td>AKT</td>
<td>Rabbit</td>
<td>CST</td>
<td>#603</td>
<td>Complementary</td>
<td>1.25</td>
<td>4 µg/ml</td>
</tr>
<tr>
<td>pMAPK</td>
<td>Rabbit</td>
<td>CST</td>
<td>#125</td>
<td>Dose and nuclear</td>
<td>1.25</td>
<td>3 µg/ml</td>
</tr>
<tr>
<td>pmTOR</td>
<td>Rabbit</td>
<td>Albumin</td>
<td>#S0004</td>
<td>Radian</td>
<td>1.50</td>
<td>10 µg/ml</td>
</tr>
<tr>
<td>Emc3h</td>
<td>Rabbit</td>
<td>CST</td>
<td>#568</td>
<td>Radian</td>
<td>1.80</td>
<td>1.5 µg/ml</td>
</tr>
<tr>
<td>Wnt8</td>
<td>Rabbit</td>
<td>Lab Vector</td>
<td>#G024-F</td>
<td>Complementary</td>
<td>1.50</td>
<td>6 µg/ml</td>
</tr>
<tr>
<td>phospho g55</td>
<td>Rabies polyclonal</td>
<td>CST</td>
<td>#207</td>
<td>Radian</td>
<td>1.50</td>
<td>4 µg/ml</td>
</tr>
<tr>
<td>GFAP</td>
<td>Mouse monoclonal</td>
<td>Santa Cruz</td>
<td>sc-56422</td>
<td>Radian</td>
<td>1.50</td>
<td>3 µg/ml</td>
</tr>
<tr>
<td>Sis</td>
<td>Rabbit</td>
<td>CST</td>
<td>#215</td>
<td>Complementary</td>
<td>1.50</td>
<td>3 µg/ml</td>
</tr>
</tbody>
</table>

(57) Abstract: Interestingly, the significant biomarkers for Gefitinib-treated GBM patients (RTOG 0211) appeared to differ compared to historical, RT and non- Gefitinib-treated GBM patients. In Gefitinib-treated patients, those with higher levels of nuclear pAKT driven by PTEN loss, higher levels of nuclear pMAPK, and lower levels of nuclear pmTOR had significantly worse clinical outcomes. In contrast, in non-Gefitinib-treated patients, patients with PTEN-deficiency, and higher levels of EGFRvIII, total EGFR, IGFRI, NFkB and lower levels of nuclear Survivin appeared to have adverse clinical outcomes, highlighting the treatment-dependency of these biomarkers.
A. CLASSIFICATION OF SUBJECT MATTER

According to International Patent Classification (IPC) or to both national classification and IPC:

INV. C12Q1/42 G01N33/574

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12Q GO1N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>TERAKAWA N ET AL: &quot;Loss of PTEN expression followed by Akt phosphorylation is a poor prognostic factor for patients with endometrial cancer&quot; ENDOCRINE-RELATED CANCER 200306 GB, vol. 10, no. 2, June 2003 (2003-06), pages 203-208, XP008116820 ISSN: 1351-0088 the whole document</td>
<td>1,4</td>
</tr>
</tbody>
</table>

Further documents are listed in the continuation of Box C

see patent family annex

Date of the actual completion of the international search: 18 January 2010

Date of mailing of the international search report: 07/04/2010

Name and mailing address of the ISA:
European Patent Office, P B 5818 Patentlaan 2 NL-2280 HV RUISWIJK Tel: (+31-70) 340-2040, Fax: (+31-70) 340-3016

Authorized officer: Gundlach, Björn

Form PCT/ISA/210 (second sheet) (April 2005)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No</th>
</tr>
</thead>
<tbody>
<tr>
<td>X</td>
<td>KANAMORI YASUNOBU ET AL: &quot;PTEN expression is associated with prognosis for patients with advanced endometrial carcinoma undergoing postoperative chemotherapy&quot; INTERNATIONAL JOURNAL OF CANCER, vol. 100, no. 6, 20 August 2002 (2002-08-20), pages 686-689, XP008116824 ISSN: 0020-7136 the whole document</td>
<td>1-4</td>
</tr>
<tr>
<td>X</td>
<td>JIANG ZIBIN ET AL: &quot;Phosphatase and tensin homologue deficiency in glioblastoma confers resistance to radiation and temozolomide that is reversed by the protease inhibitor nelfinavir&quot; CANCER RESEARCH, vol. 67, no. 9, May 2007 (2007-05), pages 4467-4473, XP008116879 ISSN: 0008-5472 the whole document</td>
<td>1-6,22</td>
</tr>
<tr>
<td>A</td>
<td>WICK WOLFGANG ET AL: &quot;PTEN gene transfer in human malignant glioma: Sensitization to irradiation and CD95L-induced apoptosis&quot; ONCOGENE, vol. 18, no. 27, 8 July 1999 (1999-07-08), pages 3936-3943, XP002283535 ISSN: 0950-9232 the whole document</td>
<td>1-6,22</td>
</tr>
</tbody>
</table>
# INTERNATIONAL SEARCH REPORT

**Box No. II  Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [ ] Claims Nos.:
   * because they relate to subject matter not required to be searched by this Authority, namely:

2. [ ] Claims Nos.:
   * because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
   * because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

**Box No. III  Observations where unity of invention is lacking (Continuation of item 3 of first sheet)**

This International Searching Authority found multiple inventions in this international application, as follows:

> see additional sheet

1. [X] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

   1-6, 22 (all partially)

**Remark on Protest**

- [ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- [ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- [ ] No protest accompanied the payment of additional search fees.

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Form PCT/ISA/210 (continuation of first sheet (2)) (April 2005)
This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-6, 22 (all partially)

   A method of determining a prognosis of a patient using PTEN as a marker, wherein high levels of PTEN indicate a poor prognosis when treated with radiation therapy alone.

2. claims: 9-21, 23-26 (all partially)

   A method of determining a prognosis of a patient using PTEN as a marker, wherein patients are not treated with radiation therapy alone.

3. claims: 1-6, 9-26 (all partially)

   A method of determining a prognosis of a patient using EGFvIII as a marker.

4. claims: 1-6, 9-27 (all partially)

   A method of determining a prognosis of a patient using EGFR as a marker.

5. claims: 1-6, 9-26 (all partially)

   A method of determining a prognosis of a patient using IGF-IR as a marker.

6. claims: 1-6, 9-27 (all partially)

   A method of determining a prognosis of a patient using NFkappaB as a marker.

7. claims: 7-26 (all partially)

   A method of determining a prognosis of a patient using pAKT as a marker.

8. claims: 7-26 (all partially)

   A method of determining a prognosis of a patient using pmTOR as a marker.

9. claims: 7-26 (all partially)
A method of determining a prognosis of a patient using pMAPK as a marker.

10. claims: 7,15-20,23,24,27 (all partially)

A method of determining a prognosis of a patient using Src as a marker.

11. claims: 9-26 (all partially)

A method of determining a prognosis of a patient using survivin as a marker.