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## (72) Inventor; and

(71) Applicant : CHENG, Ning Man [CN/CN]; Room 501,  
5/F., Henley Building, 5 Queen's Road, Central, Hong  
Kong (CN).

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(54) Title: USE OF PEGYLATED RECOMBINANT HUMAN ARGINASE FOR TREATMENT OF LEUKEMIA

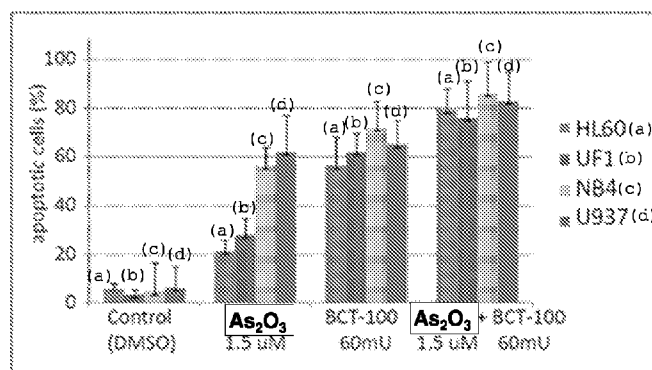


Fig. 2

(57) Abstract: The present invention provides a method for treatment of leukemia comprising administration of arginase to a subject in need thereof. In one embodiment, the leukemia is lymphocytic or myeloid. In another embodiment, the leukemia is arsenic resistant. In a further embodiment, the arginase is pegylated recombinant human arginase. In another embodiment, the arginase can be administered in combination with a second therapeutic agent such as Doxorubicin in the treatment of leukemia.

## USE OF PEGYLATED RECOMBINANT HUMAN ARGINASE FOR TREATMENT OF LEUKEMIA

**CROSS-REFERENCE TO RELATED APPLICATION**

- 5 [0001] This application claims benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application having Serial No. 61/425,243 filed on Dec. 21, 2010, which is hereby incorporated by reference herein in its entirety.

**FIELD OF INVENTION**

- 10 [0002] This invention relates to method of treating leukemia with arginase. In particular, the method relates to treatment of leukemia with pegylated recombinant human arginase.

**BACKGROUND OF INVENTION**

- 15 [0003] Haematologic malignancies, such as non-Hodgkin's lymphoma and leukemia, rank number 10 in the most common cancers worldwide. Acute lymphocytic leukemia is one of the most common pediatric malignances and remains the leading cause of death from a disease in children despite the high curing rates achieved with contemporary regimens. In adults, hemic malignancies account for about 10% of all cancers. Chemotherapy together with target therapy remains the mainstay of treatment. On relapse, 20 bone marrow transplantation offers the only means of cure. However, this modality of treatment can only be offered to patients with suitable Human leukocyte antigen (HLA) compatible donors. For the unfortunate patients with refractory leukemias and lymphomas without suitable marrow donors, prognosis is grim.

- 25 [0004] The standard of care for leukemia and lymphoma is chemotherapy given systemically and intrathecally in conjunction with various target therapies such as rituximab, anti-CD30, Campath etc. Often, radiation is employed for cranial prophylaxis and local therapy for lymphomas, in particular the Hodgkin's lymphomas. In patients with relapsed leukemia and lymphoma, infusion of HLA compatible stem cells either from related donors or unrelated donors following high-dose chemotherapy as in the case of

30 bone marrow transplantation can be curative, but with high morbidity and a modest treatment-related death. For those patients with refractory disease in absence of suitable HLA donors, no standard treatment exists. Patients can be considered for clinical trials or given palliation; in either case, prognosis is extremely poor. Therefore there is clearly a need for a new and improved treatment method.

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### SUMMARY OF INVENTION

[0005] The present invention provides a method for treating leukemia in a patient. The method involves the administration to the patient a therapeutically effective amount of a composition comprising arginase, wherein said composition is effective at treating  
40 lymphocytic and/or myeloid leukemia.

[0006] In an exemplary embodiment of the present invention, the lymphocytic leukemia is acute. In another exemplary embodiment, the lymphocytic leukemia is chronic.

[0007] In another exemplary embodiment of the present invention, the lymphocytic leukemia is T-cell acute lymphocytic leukemia.

45 [0008] In an exemplary embodiment of the present invention, the myeloid leukemia is acute. In another exemplary embodiment, the myeloid leukemia is chronic.

[0009] In yet another exemplary embodiment of the present invention, the myeloid leukemia is arsenic resistant myeloid leukemia.

[0010] In another exemplary embodiment, the arginase is pegylated recombinant human  
50 arginase. In a further embodiment, the pegylating agent is methoxy-polyethylene glycol succinimidyl propionic acid (mPEG-SPA).

[0011] In a further aspect of the present invention, a method of treating arsenic resistant leukemia is provided. In this method a therapeutically effective amount of a composition comprising arginase is administered to a patient, wherein the composition is effective at  
55 treating arsenic resistant lymphocytic and/or arsenic resistant myeloid leukemia. In an exemplary embodiment, the arginase is pegylated recombinant human arginase. In a

further exemplary embodiment, the pegylating agent is methoxy-polyethylene glycol succinimidyl propionic acid (mPEG-SPA).

[0012] The arginase of the present invention can be administered in combination with a second therapeutic agent. In an exemplary embodiment, the second therapeutic agent is Doxorubicin.

[0013] In yet a further aspect, the arginase comprising composition of the present invention is administered intravenously, intraperitoneally, subcutaneously, or intramuscularly.

### BRIEF DESCRIPTION OF FIGURES

[0014] Fig. 1a and Fig. 1b show respectively the effect of arsenic trioxide ( $\text{As}_2\text{O}_3$ ) and BCT-100 on cell viability in various promyelocytic leukemia cell lines.

[0015] Fig. 2 shows the effect of BCT-100 and arsenic trioxide in inducing apoptosis in various promyelocytic leukemia cell lines.

[0016] Fig. 3 shows BCT-100 induces apoptosis via inhibition of pmTOR and induction of Stat3 and Bax.

[0017] Fig. 4a shows the induction of granulocytic differentiation in NB4 by BCT-100. Fig. 4b shows the BCT-100 induced differentiation in NB4 and HL60 cells. Fig 4c shows the granulocytic morphology of BCT-100 treated NB4 cells.

[0018] Fig. 5a and Fig 5b show the reorganization of promyelocytic leukemia nuclear bodies in HL60 cells after BCT-100 treatment

[0019] Fig. 6 shows the  $\text{IC}_{50}$  of BCT-100 in various cancer cell lines.

[0020] Fig. 7 shows the effect of BCT-100 on various leukemia cell lines when administered in combination with Doxorubicin.

[0021] Fig. 8 shows the effect of BCT-100 on the liver, spleen and sternum cytology of HL60-incoluated NOD/SCIDS mice.

[0022] Fig. 9 shows the enhanced survival rate of HL60-incoluated NOD/SCIDS mice  
85 when treated with BCT-100.

### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0023] As used herein and in the claims, “comprising” means including the  
90 following elements but not excluding others.

[0024] The present invention provides the use of arginase in treating leukemia. In certain embodiments, pegylated recombinant human arginase is used for the treatment of various types of leukemia. In a further embodiment, the pegylated recombinant human arginase is BCT-100 and the preparation of pegylated recombinant human arginase and the steps for  
95 pegylating the same are disclosed in, e.g., US 10/518,223, which is incorporated in its entirety by reference.

[0025] Arginase is a manganese-containing enzyme, catalyzing the conversion of arginine to ornithine and urea, the last step of the Urea Cycle. Pegylated recombinant human arginase in our preclinical study showed efficacious in inducing arginine depletion in  
100 dose-dependent manner.

[0026] The present invention also provides the use of arginase, for example, pegylated recombinant human arginase such as BCT-100 in treating cancer in a patient who suffers from refractory or relapsed leukemia or lymphoma. The term “refractory or relapsed leukemia or lymphoma” refers to the condition of a patient suffering from leukemia or  
105 lymphoma that the patient is unresponsive to all available drugs for treating leukemia or lymphoma, or signs and symptoms return to the patients upon using all other available drugs for treating leukemia or lymphoma.

[0027] The present invention shows that arginase, for example, pegylated recombinant human arginase such as BCT-100 is at least 6-10 times more effective in treating T-cell leukemia and myeloid leukemia than treating hepatocellular carcinoma.

[0028] The arginase of the present invention is shown to be effective in treating various types of leukemia, e.g., lymphocytic leukemia including but not limited to T-cell leukemia; myeloid leukemia including but not limited to promyelocytic leukemia.

[0029] The arginase of the present invention can be administrated in combination with a second therapeutic agent such as Doxorubicin. In various embodiments, enhanced therapeutic effect was observed when BCT-100 was administrated in combination with Doxorubicin.

[0030] The present invention also shows that arginase, for example, pegylated recombinant human arginase such as BCT-100 is effective in treating both arsenic sensitive and arsenic resistant leukemia. The effectiveness of BCT-100 in inducing apoptosis in both arsenic sensitive and arsenic resistant leukemia is also demonstrated in the present invention.

[0031] The present invention is further defined by the following examples, which are not intended to limit the present invention. Reasonable variations, such as those understood by reasonable artisans, can be made without departing from the scope of the present invention.

[0032] Example 1

[0033] The Inhibitory Effect Of BCT-100 On Various Leukemia Cell Lines

[0034] The inhibitory effect of BCT-100 in acute myeloid leukemia cells (Kasumi-1a, ML2, HL60, K562 and NB4) and T-cell leukemia cells (Jurkat, ALL-SIL, HPB-ALL and TALL-1) was studied. The  $IC_{50}$  values of BCT-100 in various cell lines are shown in Table 1. The result indicates that BCT-100 is effective in inhibiting the growth of leukemia, including myeloid leukemia and lymphocytic leukemic.

Table 1 - IC<sub>50</sub> of BCT-100 in various leukemia cell lines.

| Myeloid Leukemia | IC <sub>50</sub> (mU/mL) | T-cell Leukemia | IC <sub>50</sub> (mU/mL) |
|------------------|--------------------------|-----------------|--------------------------|
| Kasumi-1a        | 65                       | Jurkat          | 40                       |
| ML2              | 65                       | All-SIL         | 120                      |
| HL60             | 55                       | HPB-ALL         | 110                      |
| K562             | 25                       | TALL-1          | 100                      |
| NB4              | 60                       |                 |                          |

## 135 [0035] Example 2

## [0036] Effect of BCT-100 On Arsenic Sensitive and Arsenic Resistant Myelocytic Cell Lines

The effect of BCT-100 on arsenic sensitive myelocytic cell lines (NB4 and U937) as well as arsenic resistant myelocytic cell lines (HL60 and UF1) was investigated. Fig. 1a shows the effect of arsenic trioxide on cell viability in myelocytic leukemia cells. The cell viability in the arsenic sensitive NB4 and U937 cell lines dropped as the concentration of arsenic increased. However, HL60 and UF1 cell lines did not respond to As<sub>2</sub>O<sub>3</sub> treatment.

[0037] Fig. 1b shows the effect of BCT-100 on cell viability in myelocytic leukemia cells NB4, U937, HL60 and UF1. Both the arsenic sensitive and resistant leukemia cells responded to the BCT-100 treatment. In all cases the cell viability dropped with increasing amount of BCT-100 in the medium.

[0038] The results showed BCT-100 is effective in inhibiting the growth of myelocytic leukemia, including myelocytic leukemia that is resistant to arsenic. Therefore, the arginase of the present invention is useful for treating arsenic resistant leukemia.

## 150 [0039] Example 3

## [0040] Effect of BCT-100 In Inducing Apoptosis In Leukemia Cells

[0041] The effect of BCT-100 in inducing apoptosis in both arsenic sensitive (NB4 and U937) and arsenic resistant (HL60 and UF1) leukemia cell lines was tested. As shown in

Fig. 2, arsenic was effective in inducing apoptosis in leukemic cell lines NB4 and  
155 U937. However, the apoptosis rate was low in HL60 and UF1 cells in the presence of  
arsenic, as these cell lines are arsenic resistant. BCT-100 was found to be effective in  
inducing apoptosis in both arsenic sensitive and arsenic resistant leukemia cell lines. The  
apoptosis rate was further enhanced when BCT-100 is administrated in combination with  
arsenic trioxide.

160 [0042] Example 4

[0043] Mechanism Of BCT-100 Induced Apoptosis

[0044] Expression of Bax and pmTOR was assessed in leukemic cell line HL60 by  
western blotting with or without BCT-100 treatment using monoclonal antibodies against  
Bax and pmTOR respectively. The same blot was stripped and probed with anti-actin  
165 antibody for loading control. Apoptosis was determined by annexin V labeling. HL60  
cells were cultured in medium with or without BCT-100 and the expression of annexin V  
were analyzed by flow-cytometry at 8, 16, 24, and 36 hrs after BCT-100 treatment.

[0045] As revealed by western blot analysis shown in Fig. 3, the Bax level was up-  
regulated 8 hrs after BCT-100 treatment and remained high levels afterwards. The protein  
170 level of pmTOR was down-regulated in response to BCT-100 treatment. These results  
indicate that BCT-100 may induce apoptosis of leukemic cell line HL60 through signal  
transduction pathway involving Bax/Bcl-2 or specifically due to inhibition of pmTOR  
signaling.

[0046] Example 5

175 [0047] Induction Of Granulocytic Differentiation By BCT-100

[0048] The induction of granulocytic differentiation in NB4 and HL60 cells was studied.  
Fig. 4a shows that BCT-100 induced granulocytic differentiation in NB4 cells. As  
revealed by fluorescence-activated cell sorting analysis of CD11b expression shown in  
Fig. 4b, BCT-100 induced granulocytic differentiation in NB4 and HL60 cells within 96-

180 hour treatment with BCT-100. Fig. 4c shows the granulocytic morphology of NB4 cells which were treated with BCT-100. Specifically, decrease in nuclear to cytoplasmic ratio, appearance of cytoplasmic granules, chromatin condensation and loss of nucleoli were observed.

[0049] Example 6

185 [0050] Reorganization Of Promyelocytic Leukemia Nuclear Bodies

[0051] The reorganization of promyelocytic leukemia nuclear bodies in HL60 cells was studied after treatment with BCT-100. Immunostaining with anti-promyelocytic leukemia antibodies revealed a diffusely microspeckled pattern of promyelocytic leukemia in the nuclei of control (DMSO treated) HL60 cells as shown in Fig. 5a. In cells treated with  
190 BCT-100, the microspeckled pattern disappeared and the size and brightness of the promyelocytic leukemia bodies returned to normal.

[0052] Example 7

[0053] IC<sub>50</sub> of BCT-100 In Various Cancer Cell Lines

[0054] The IC<sub>50</sub> values of BCT-100 in various cancer cell lines were investigated. The  
195 cancer cell lines being tested were T-cell acute lymphocytic leukemia, acute myeloid leukemia, hepatocellular carcinoma and pancreatic cancer. The results in Fig. 6 showed that BCT-100 is effective in treating T-cell acute lymphocytic leukemia, acute myeloid leukemia, hepatocellular carcinoma and pancreatic cancer. Further, BCT-100 was found to be at least 6-10 times more potent in treating leukemia and pancreatic cancer than treating  
200 hepatocellular carcinoma.

[0055] Example 8

[0056] Effect of BCT-100 On Various Leukemia Cell Lines When Administrated In Combination With Doxorubicin

[0057] The combination effect of BCT-100 with Doxorubicin on various leukemia cell lines was studied. The leukemia cell lines tested were myeloid leukemia cell lines Kasumi-1a, ML2, HL60, K562, NB4 and lymphocytic leukemia cell lines ALL-SIL and Jurkat. Referring to Figure 7, both BCT-100 and Doxorubicin were found to be effective in treating leukemia, including myeloid and lymphocytic leukemia, when administrated alone respectively. Enhanced therapeutic effect was observed when BCT-100 was administrated in combination with Doxorubicin. The enhancement effect was highly noticeable in all myeloid leukemia cell lines as well as ALL-SIL. The result showed BCT-100 can be administrated with Doxorubicin for the treatment of leukemia, in particular myeloid leukemia and/or lymphocytic leukemia.

[0058] Example 9

[0059] Effect Of BCT-100 On The Liver, Spleen And Sternum Cytology Of HL60-Incoluated NOD/SCIDS Mice

[0060] HL60 cells were injected to the tail of NOD/SCIDS mice. Treatment commenced on day 14. The animals were divided into 4 groups. The Doxorubicin group received treatment by doxorubicin, which was administrated at 3mg/kg/day by intraperitoneal injection 3 times weekly for the first week, thereafter once per week for 4 weeks. The BCT-100 group received treatment by BCT-100, which was administrated at 50U/mice by intraperitoneal injection weekly for 4 weeks. The combinational group received treatment of both Doxorubicin and BCT-100, administrated as described above. The control group received intraperitoneal injection of saline. Figure 8 shows the liver, spleen and sternum cytology of the 4 groups of mice after treatment. In the control group, infiltration of leukemia in liver, spleen and sternum was observed. Modest infiltration was observed in the Doxorubicin and BCT-100 group in the liver, spleen and sternum. In the combinational group, note regression of blast from liver and near-normal morphology of sternum was observed.

230 [0061] The result shows that BCT-100 is effective in clearing leukemic blasts in the sternal bone marrow, spleen and liver. BCT-100 can also be administrated in combination with Doxorubicin to enhance the therapeutic effect.

[0062] Example 10

235 [0063] Enhanced Survival Rate of HL60-incoluated NOD/SCIDS Mice When Treated With BCT-100

[0064] The effect of BCT-100 on the survival rate of HL-60-incoluated NOD/SCIDS mice was investigated. Referring to Figure 9, the survival rate and survival days of mice treated with BCT-100 are increased as compared to the control group, indicating that BCT-100 alone is effective in treating leukemia, e.g., myeloid leukemia. The survival rate  
240 and days are further enhanced when BCT-100 is administrated with Doxorubicin. Thus BCT-100 can be administrated with Doxorubicin to treat leukemia, e.g., myeloid leukemia.

[0065] The exemplary embodiments of the present invention are thus fully described. Although the description referred to particular embodiments, it will be clear to one skilled  
245 in the art that the present invention may be practiced with variation of these specific details. Hence this invention should not be construed as limited to the embodiments set forth herein.

[0066] Reference

1. Savoca KV, Davis FF, van Es T, McCoy JR, Palczuk NC. Cancer therapy with  
250 chemically modified enzymes. II. The therapeutic effectiveness of arginase, and arginase modified by the covalent attachment of polyethylene glycol, on the taper liver tumor and the L5178Y murine leukemia. Cancer Biochem Biophys 1984;7:261–8

- 255 2. L Scott, J Lamb, S Smith and DN Wheatley, Single amino acid (arginine) deprivation:  
rapid and selective death of cultured transformed and malignant cells, British Journal  
of Cancer 2000; 83(6), 800–810
3. Storr JM, Burton AF. The effects of arginine deficiency on lymphoma cells. Br J  
Cancer 1974;30:50–9
- 260 4. Denys N Wheatley, Elaine Campbell, Paul BS Lai and Paul NM Cheng. A rational  
approach to the systemic treatment of cancer involving medium-term depletion of  
arginine, Gene Therapy Molecular Biology 2005; Vol 9, 33-40
5. Osunkoya BO, Adler WH & Smith RT, Effect of Arginine deficiency on synthesis of  
DNA and Immunoglobulin receptor of Burkitt Lymphoma cells. Nature 1970; 227, 398  
- 399
- 265 6. H Gong, F Zolzer, G von Recklinghausen, W Havers and L Schweigerer, ADI inhibits  
proliferation of human leukemia cells more potently than asparaginase by inducing  
cell cycle arrest and apoptosis. Leukemia 2000; 14, 826-829
- 270 7. Cheng PN et al. Remission of hepatocellular carcinoma with arginine depletion  
induced by systemic release of endogenous hepatic arginase due to transhepatic  
arterial embolisation, augmented by high-dose insulin: arginase as a potential drug  
candidate for hepatocellular carcinoma, Cancer Letters 2005; 224, 67-80.
8. Cheng PN et al. Pegylated Recombinant Human Arginase (rhArg-peg5,000mw)  
Inhibits the *In vitro* and *In vivo* Proliferation of Human Hepatocellular Carcinoma  
through Arginine Depletion, Cancer Research 2007; 67: (1).
- 275 9. T L Lam, G K Y Wong, H C Chong, P N M Cheng, S C Choi, S Y Kwok, R T P Poon,  
D N Wheatley, W H Lo, Y C Leung (2009) Recombinant human arginase inhibits  
proliferation of human hepatocellular carcinoma by inducing cell cycle arrest, Cancer  
Letters 2009; 277 (1): 91-100

10. Sam-Mui Tsui; Wai-Man Lam; Tin-Lun Lam; Hiu-Chi Chong; Pui-Kin So; Sui-Yi  
280 Kwok; Simon Arnold; Paul Ning-Man Cheng; Denys Wheatley; Wai-Hung Lo and  
Yun-Chung Leung. Pegylated derivatives of recombinant human arginase (rhArg) for  
sustained *in vivo* activity in cancer therapy: preparation, characterization and analysis  
of their pharmacodynamics *in vivo* and *in vitro* action upon hepatocellular carcinoma  
cell (HCC), Cancer Cell International 2009, 9:9

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What is claimed is:

1. A method of treating leukemia in a patient comprising administering to the patient a therapeutically effective amount of a composition comprising arginase, wherein said composition is effective at treating lymphocytic and/or myeloid leukemia.
2. The method of claim 1 wherein said lymphocytic leukemia is acute lymphocytic leukemia or chronic lymphocytic leukemia.
3. The method of claim 1 wherein said lymphocytic leukemia is T-cell acute lymphocytic leukemia.
4. The method of claim 1 wherein said myeloid leukemia is acute myeloid leukemia or chronic myeloid leukemia.
5. The method of claim 1 wherein said myeloid leukemia is arsenic resistant myeloid leukemia.
6. The method of claim 1 wherein said leukemia is arsenic resistant.
7. The method of claim 1 wherein said arginase is pegylated recombinant human arginase
8. The method of claim 7 wherein said arginase is pegylated with methoxy-polyethylene glycol succinimidyl propionic acid.
9. The method of any one of claims 1-8 further comprising administering a second therapeutic agent, wherein said second therapeutic agent is doxorubicin.
10. The method of claim 1, wherein said composition is administered intravenously, intraperitoneally, subcutaneously, or intramuscularly.
11. A method of treating cancer in a patient comprising administering to the patient a therapeutically effective amount of a composition comprising arginase, wherein said patient suffers from relapsed or refractory leukemia or lymphoma.
12. The method of claim 11 wherein said leukemia or lymphoma is arsenic resistant.
13. The method of claim 11 wherein said arginase is pegylated recombinant human arginase.
14. The method of claim 13 wherein said arginase is pegylated with methoxy-polyethylene glycol succinimidyl propionic acid.

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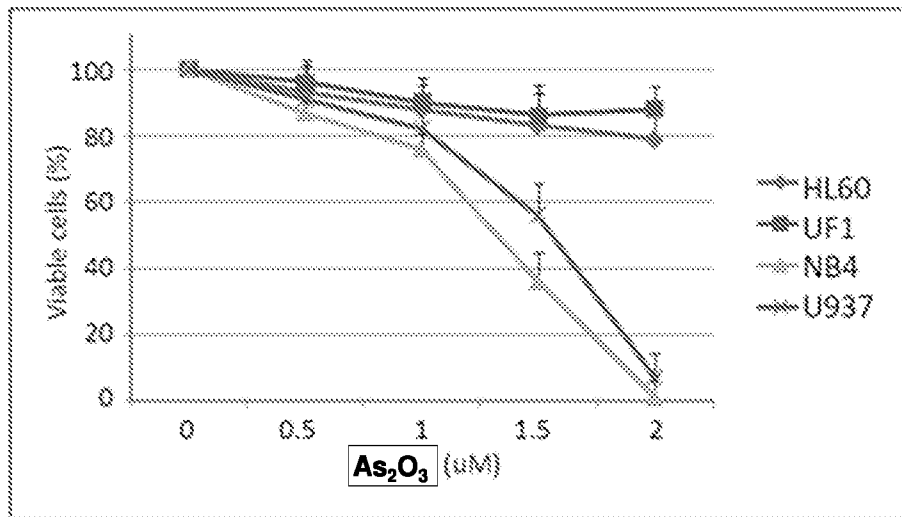


Fig. 1a

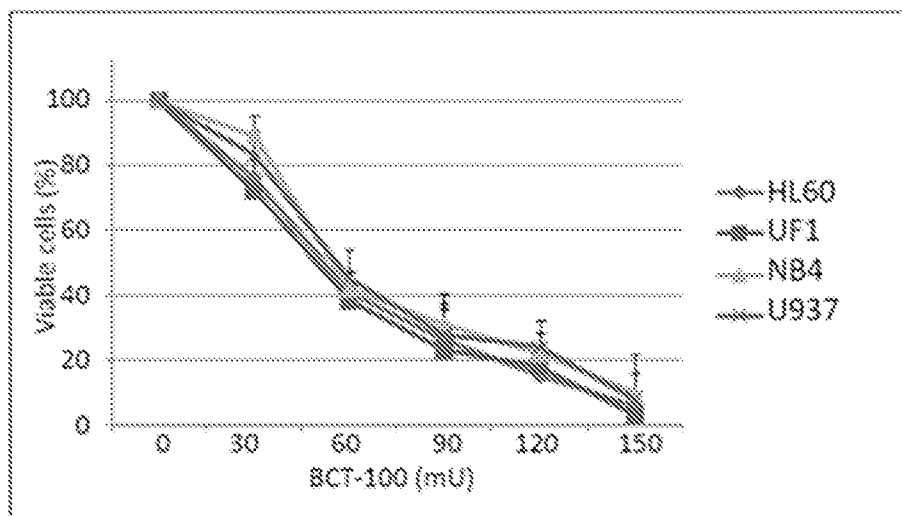


Fig. 1b

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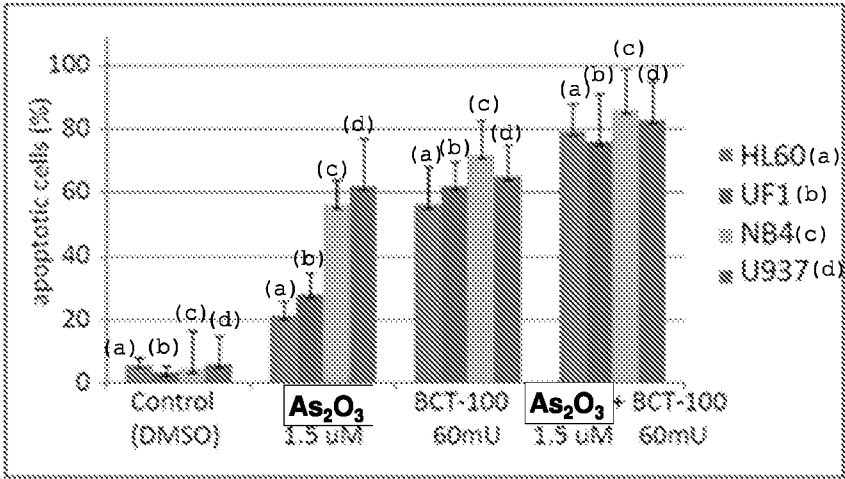


Fig. 2

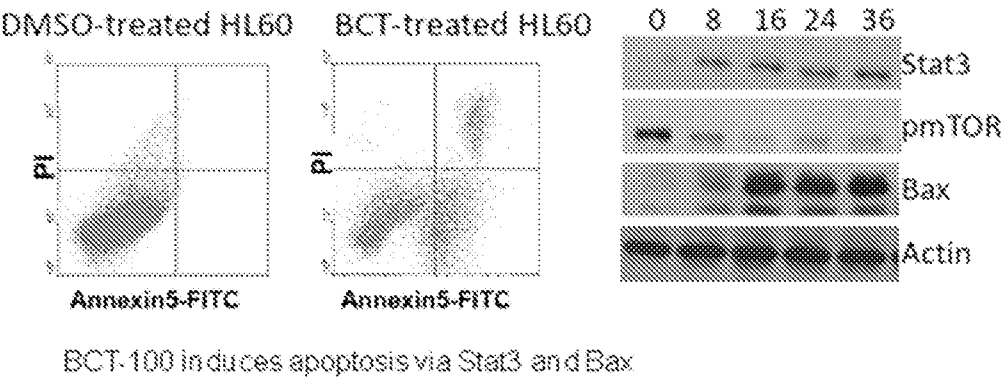


Fig. 3

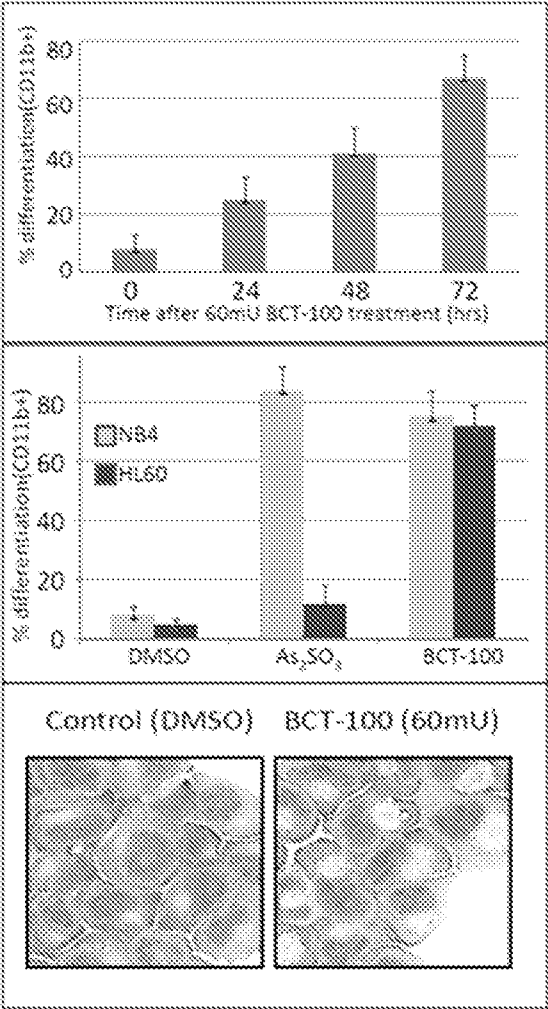


Fig. 4a

Fig. 4b

Fig. 4c

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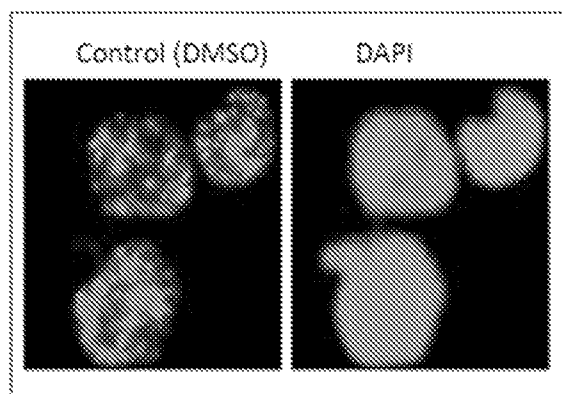


Fig. 5a

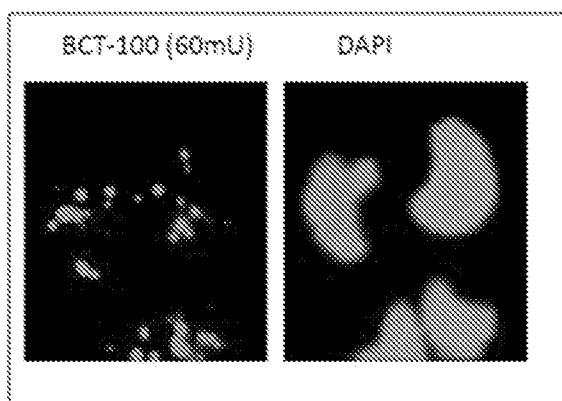


Fig. 5b

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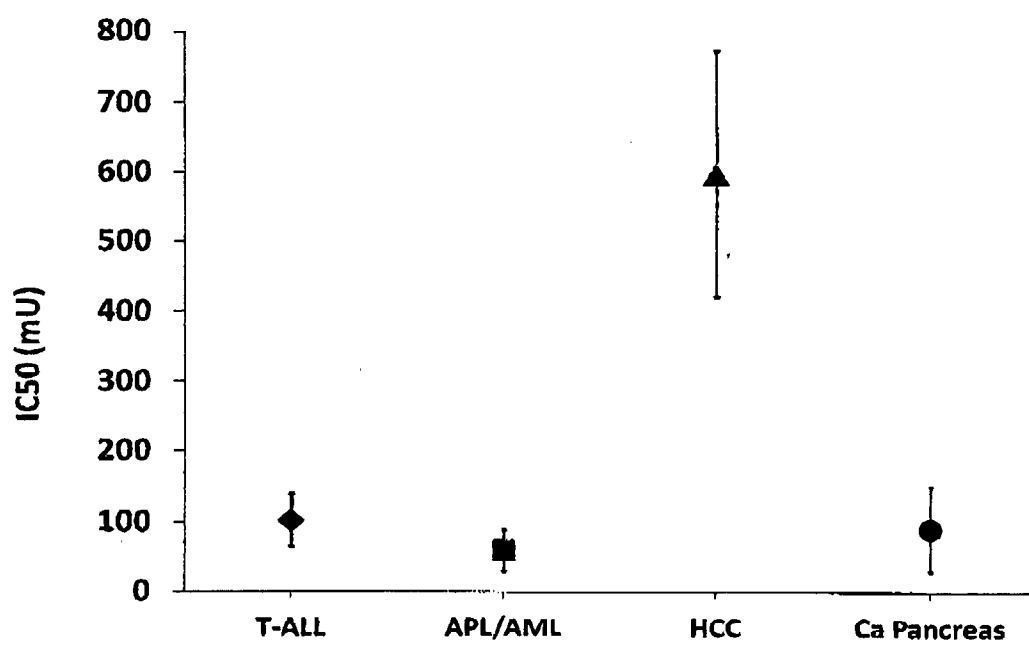


Fig. 6

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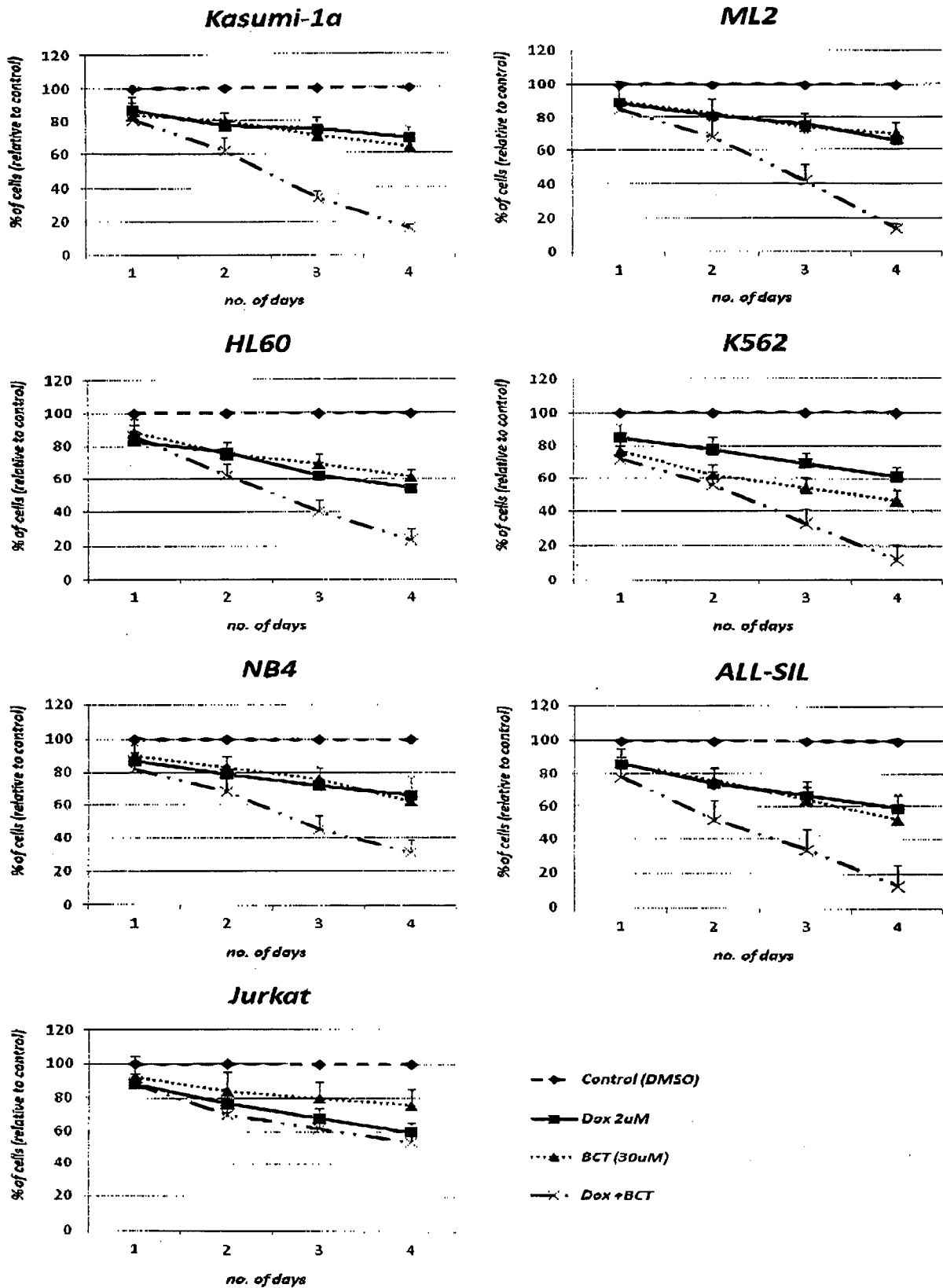


Fig. 7

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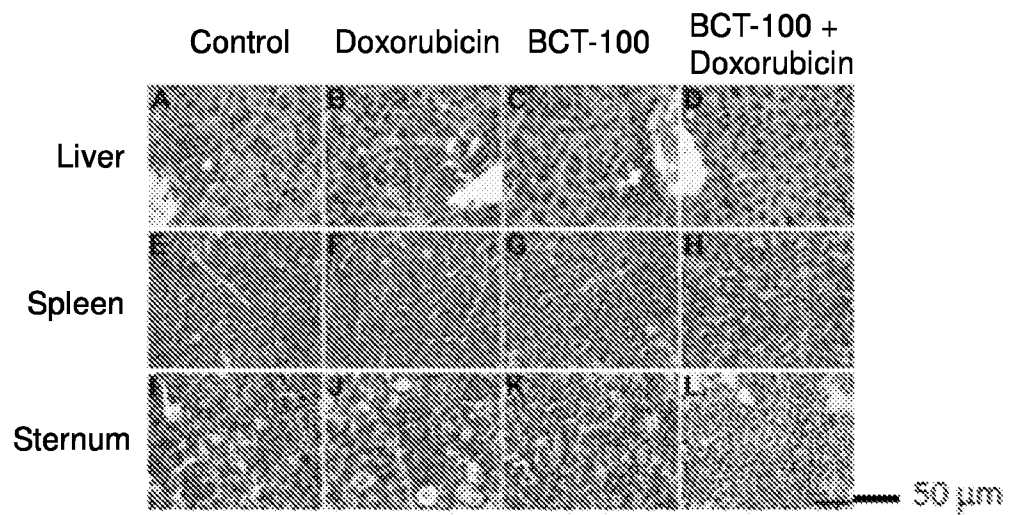


Fig. 8

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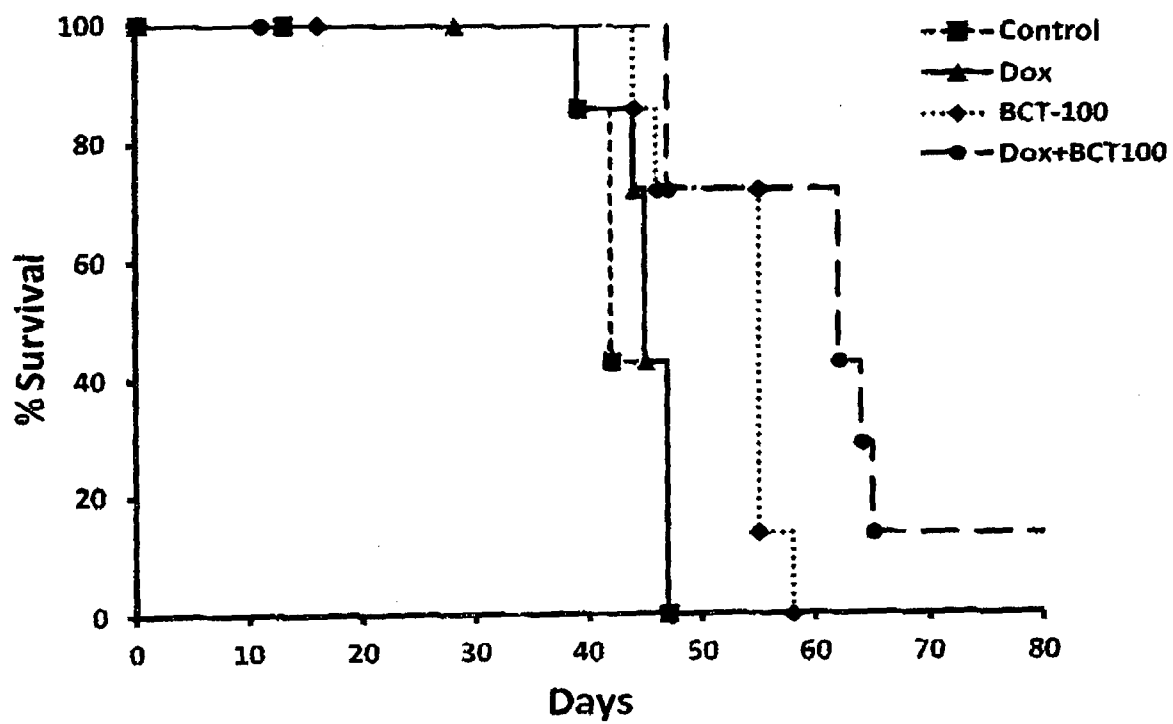


Fig. 9

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2011/055735

## A. CLASSIFICATION OF SUBJECT MATTER

See extra sheet

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

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC:A61K 38-,A61K 47-,A61P 35-

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 practicable, search terms used)

CNPAT, CNKI, WPI, EPODOC, GOOGLE, ISI web of knowledge: arginase, leukemia, pegylated

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                   | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | HERMANDEZ, Claudia P. et al. Pegylated arginase I: a potential therapeutic approach in T-ALL. BLOOD. 24 June 2010, vol. 115, No. 25, pages 5214-5221, ISSN 0006-4971 | 1-3, 6-14             |
| A         | WO2004000349 A1(BIO-CANCER TREATMENT INTERNATIONAL LIMITED)<br>31 Dec. 2003(31.12.2003) the whole document                                                           | 1-14                  |

☐ Further documents are listed in the continuation of Box C.☒ See patent family annex.

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| “P” document published prior to the international filing date but later than the priority date claimed                                                                   |                                                                                                                                                                                                                                                  |

Date of the actual completion of the international search  
05 Apr. 2012(05.04.2012)Date of mailing of the international search report  
**03 May 2012 (03.05.2012)**Name and mailing address of the ISA/CN  
The State Intellectual Property Office, the P.R.China  
6 Xitucheng Rd., Jimen Bridge, Haidian District, Beijing, China  
100088  
Facsimile No. 86-10-62019451Authorized officer  
**LV, Maoping**  
Telephone No. (86-10)62414137

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2011/055735

### 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.: 1-14

because they relate to subject matter not required to be searched by this Authority, namely:

Claims 1-14 are attributed to the methods for treatment of the human or animal body by therapy. They do not meet the criteria set out in Rules 39.1(iv) PCT.

The search report is made on the basis of the following subject-matter for claims 1-14: the use of the composition comprising arginase in the manufacture of medicament for the treatment of lymphocytic and/or myeloid leukemia.

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:

1. ☐ 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 fee.
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. ☐ 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.:

#### 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.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB2011/055735

### CLASSIFICATION OF SUBJECT MATTER:

A61K 38/50(2006.01)i

A61K 47/48(2006.01)i

A61P 35/02(2006.01)i

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
PCT/IB2011/055735

| Patent Documents referred<br>in the Report | Publication Date | Patent Family     | Publication Date |
|--------------------------------------------|------------------|-------------------|------------------|
| WO2004000349 A1                            | 31.12.2003       | AU2003250371 A1   | 06.01.2004       |
|                                            |                  | EP1517699 A1      | 30.03.2005       |
|                                            |                  | KR20050024361A1   | 10.03.2005       |
|                                            |                  | US2005244398A1    | 03.11.2005       |
|                                            |                  | JP2005538709A     | 22.12.2005       |
|                                            |                  | MXPA04012970A     | 01.09.2005       |
|                                            |                  | INMUMNP200500052E | 07.10.2005       |
|                                            |                  | NZ537774A         | 26.01.2007       |
|                                            |                  | CN1918298A        | 21.02.2007       |
|                                            |                  | EP1517699B1       | 13.02.2008       |
|                                            |                  | EP1908478A1       | 09.04.2008       |
|                                            |                  | DE60319083E       | 27.03.2008       |
|                                            |                  | MX247926B         | 08.08.2007       |
|                                            |                  | US2008226617A     | 18.09.2008       |
|                                            |                  | RU2335539C2       | 10.10.2008       |
|                                            |                  | DE60319083T2      | 05.02.2009       |
|                                            |                  | US2009123450A1    | 14.05.2009       |
|                                            |                  | CN101433714A      | 20.05.2009       |
|                                            |                  | AU2003250371B2    | 29.01.2009       |
|                                            |                  | TW200509962A      | 16.03.2005       |
|                                            |                  | IN207895B         | 10.08.2007       |
|                                            |                  | EP1908478B1       | 24.03.2010       |
|                                            |                  | DE60331871E       | 06.05.2010       |
|                                            |                  | JP2010148509A     | 08.07.2010       |
|                                            |                  | IL165858A         | 30.12.2010       |
|                                            |                  | US7951366B2       | 31.05.2011       |
|                                            |                  | CN1918298B        | 29.06.2011       |
|                                            |                  | TW331531B1        | 11.10.2010       |