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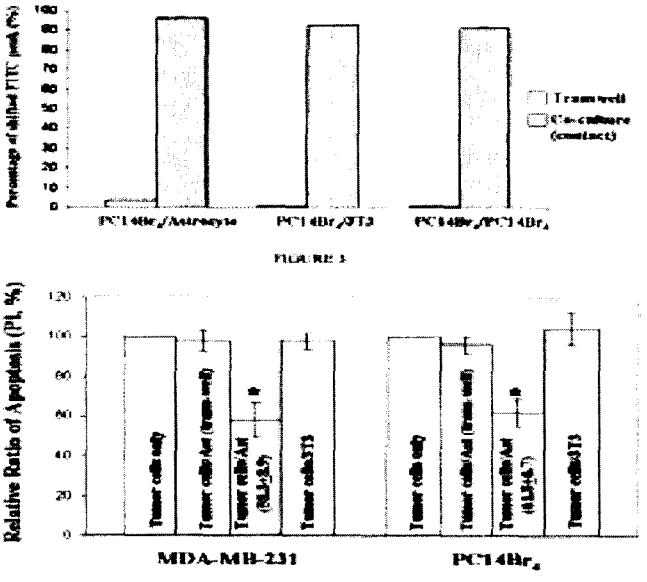
ABSTRACT

TREATMENT OF BRAIN METASTASES WITH INHIBITORS OF ENDOTHELIN RECEPTORS IN COMBINATION WITH A CYTOTOXIC CHEMOTHERAPY AGENT

The disclosure relates to an endothelin receptor antagonist for use in the prevention or treatment of brain metastases in combination with a cytotoxic chemotherapy agent, radiotherapy or both. The endothelin receptor antagonist may for example be bosentan, macitentan or a mixture of bosentan and macitentan.



I/WE CLAIM:

1. An endothelin receptor antagonist for use in the prevention or treatment of brain metastases in combination with a cytotoxic chemotherapy agent, radiotherapy or both.

2. An endothelin receptor antagonist for use in the prevention or treatment of brain metastases according to claim 1, which is a dual endothelin receptor antagonist.

3. An endothelin receptor antagonist for use in the prevention or treatment of brain metastases according to claim 1 or 2, which is selected from the group consisting of bosentan, macitentan and a mixture of bosentan and macitentan.

4. An endothelin receptor antagonist for use in the prevention or treatment of brain metastases according to one of claims 1 to 3, whereby the endothelin receptor antagonist is selected from the group consisting of bosentan, macitentan and a mixture of them and is intended to be used with a cytotoxic chemotherapy agent.

5. An endothelin receptor antagonist for use in the prevention or treatment of brain metastases according to claim 4, wherein the cytotoxic chemotherapy agent is selected from the group consisting of paclitaxel, temozolomide and a mixture of paclitaxel and temozolomide.

6. An endothelin receptor antagonist for use in the prevention or treatment of brain metastases according to claim 4, wherein the endothelin receptor antagonist is selected from the group consisting of bosentan, macitentan and a mixture of bosentan and macitentan.

7. A method of inhibiting an astrocyte mediated protection of a brain metastasis cell from a cytotoxic chemotherapy induced cell death, the method comprising the step of administering an effective amount of an endothelin receptor antagonist to the brain metastasis cell and the astrocyte to inhibit the astrocyte mediated protection.

8. A method of inhibiting an astrocyte mediated protection of a brain metastasis cell from a cytotoxic chemotherapy induced cell death, the method comprising the step of administering an effective amount of an endothelin receptor antagonist to the brain metastasis cell and the astrocyte to inhibit the astrocyte mediated protection

and further to super-sensitize the brain metastasis cell to the cytotoxic chemotherapy induced cell death.

9. The method of claim 7 or 8, further comprising the step of administering at least one cytotoxic chemotherapeutic agent to the brain metastasis cell.

10. The method of claim 9, wherein the brain metastasis cell is comprised in either an existing brain metastasis tumor in a subject or the brain metastasis cell is an isolated cell.

11. The method of claim 9 or 10 wherein the cytotoxic chemotherapy agent is at least one of paclitaxel, doxorubicin, vinblastine, vincristine, 5-fluoro-uracil, cisplatin, cyclophosphamide, etoposide, teniposide, mitomycin, irinotecan, vinorelbine, ifosfamide, and/or temozolomide.

12. The method of one of claims 7 to 11 wherein the endothelin receptor antagonist is one ore more of macitentan, sitaxentan, tezosentan, clazosentan, abbrisentan, bosentan and/or atrasentan.

13. The method of claim 11 or 12, wherein the subject is a human and optionally further comprising a step of administering a standard of care palliative and/or therapeutic treatment of the existing brain metastasis tumor in the human subject.

14. The method of claim 13, wherein the standard of care therapeutic treatment is given and the standard of care therapeutic treatment is one or both of whole brain radiotherapy or stereotactic radiosurgery.

15. A method of treating an existing brain metastasis tumor in a subject comprising administering to said subject a combination of an endothelin receptor antagonist and at least one cytotoxic chemotherapy agent.

16. A mouse having a human cancer metastasis cell brain tumor, the mouse further comprising a) at least one cytotoxic chemotherapeutic agent in a therapeutically effective amount to treat the human cancer metastasis brain tumor and b) at least one endothelin receptor antagonist in an amount sufficient to inhibit an astrocyte mediated protection of the human cancer metastasis brain tumor.

17. An isolated astrocyte cell-cancer cell complex wherein an isolated astrocyte cell is in gap junction communication with an isolated cancer cell.

18. The isolated astrocyte cell-cancer cell complex of claim 17 wherein the isolated astrocyte cell is a murine astrocyte and the isolated cancer cell is a human cancer cell.

19. The isolated astrocyte cell-cancer cell complex of claim 18, wherein the isolated cancer cell line cell is MDA-MB-231 or PC14Br4.

20. A method of forming an isolated astrocyte cell-cancer cell complex comprising the steps of a) providing an isolated astrocyte cell, b) providing a cancer cell, c) co-culturing the provided cells for a time sufficient for a gap junction form between the cells thereby forming the astrocyte cell-cancer cell complex.

21. The method of claim 20 wherein the isolated astrocyte cell is a murine astrocyte and the isolated cancer cell is a human cancer cell.

22. The method of claim 21 wherein the isolated cancer cell line is MDA-MB-231 or PC14Br4.

23. The method of claim 20, 21 or 22, further comprising the step of adding one or more candidate chemotherapy agents to assess the degree of astrocyte mediated protection against the cytotoxic effects of the chemotherapy agents.

24. The method of claim 23, further comprising the step of performing a molecular diagnostic step to provide an identifying molecular profile corresponding to the astrocyte mediated protection against the cytotoxic effects of the chemotherapy agent.

25. The method of claim 24 wherein the molecular diagnostic step is one or more of a differential gene expression measurement or a differential cellular protein concentration measurement.

26. The method of claim 25 wherein the differential gene expression measurement is performed using a gene chip.

Date **10 February 2012**

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To
The Controller of Patents
The Patent Office at New Delhi

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FIGURE 1

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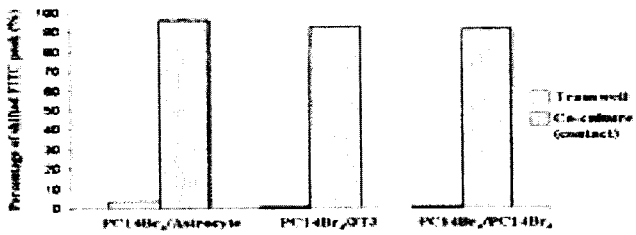

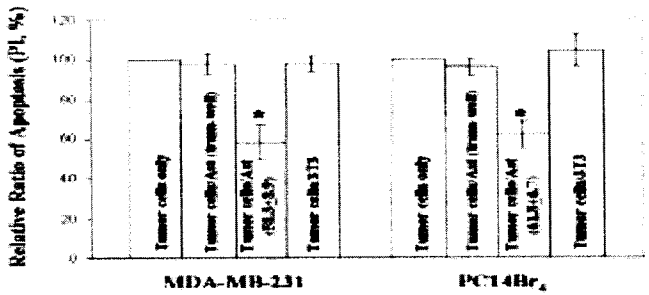

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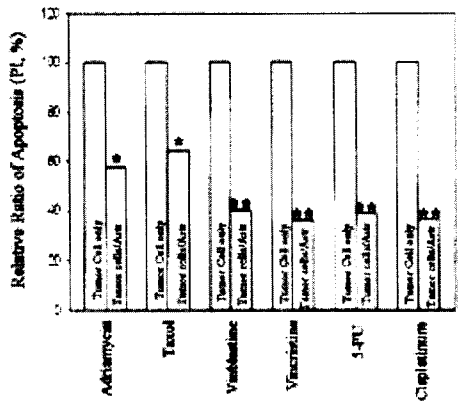

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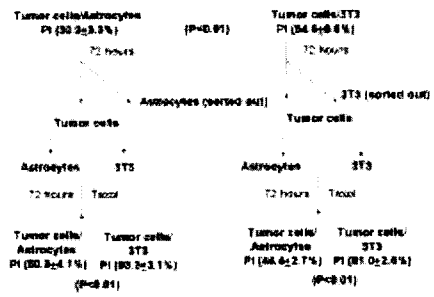
FIGURE 5

ASTROCYTE MEDIATED PROTECTION OF TUMOR CELLS FROM CHEMOTHERAPY



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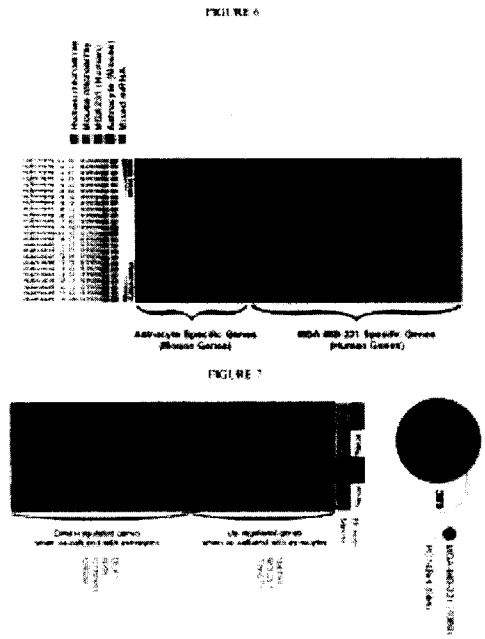

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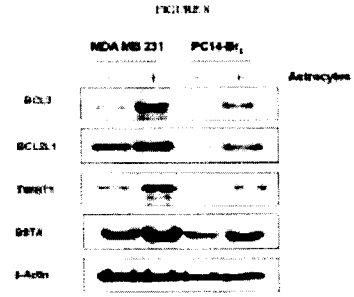

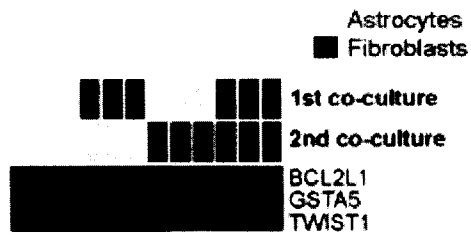

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FIGURE 10

Increased expression of ET_A-R in MDA231 human breast cancer cells co-cultured with astrocytes but not with fibroblasts (3T3)

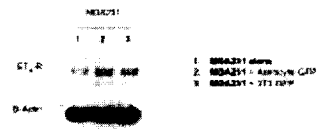

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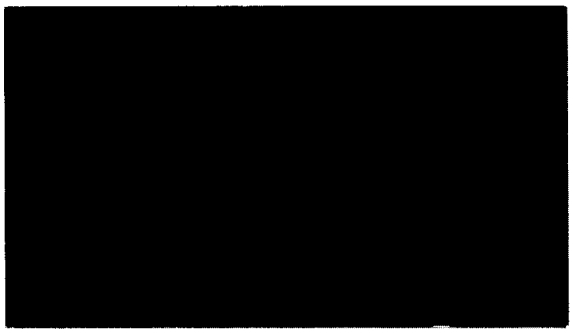
FIGURE 11

Expression of pAKT by MDA231 human breast cancer cells co-cultured with astrocytes/Taxol



Green = MDA231
Red = pAkt
Blue = nucleus

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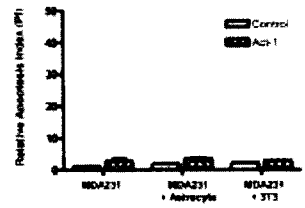

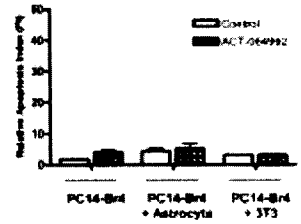

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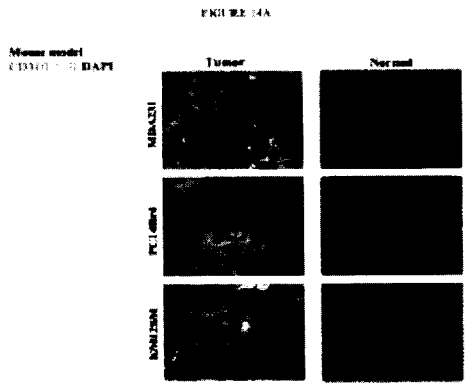

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FIGURE 14B

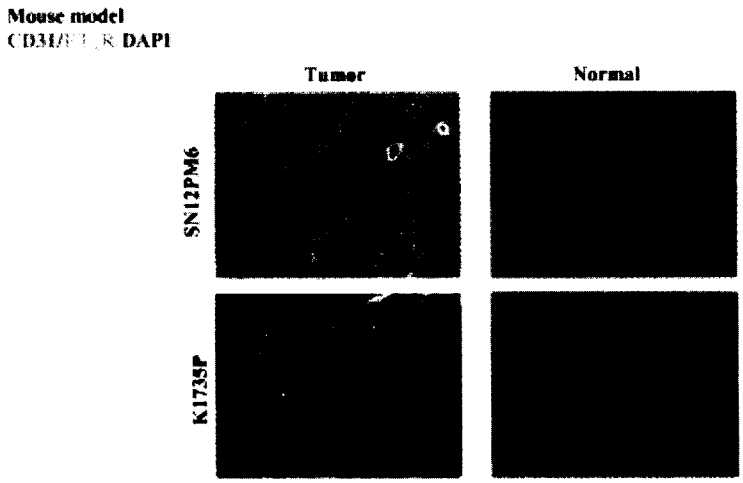

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FIGURE 14C

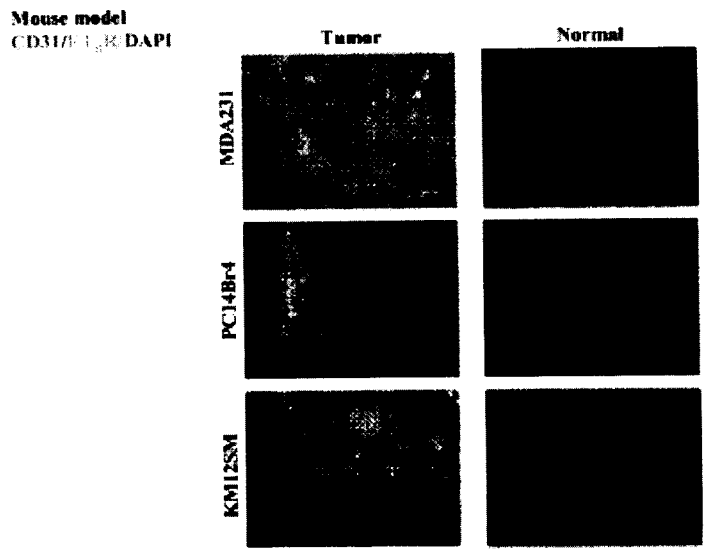

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FIGURE 14D

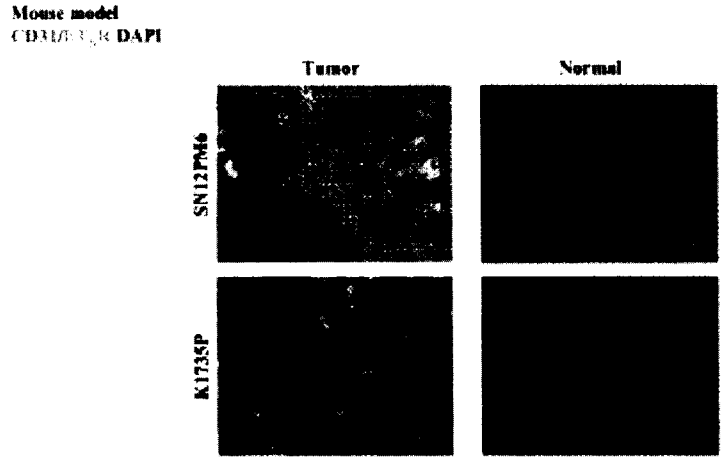

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FIGURE 15

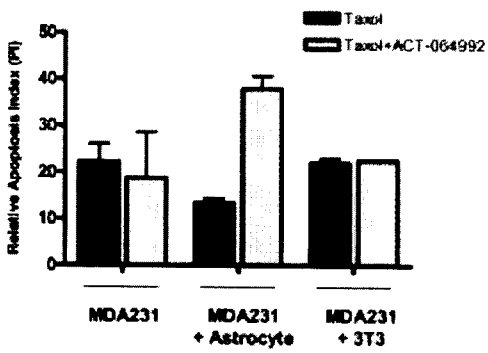

FIGURE 16

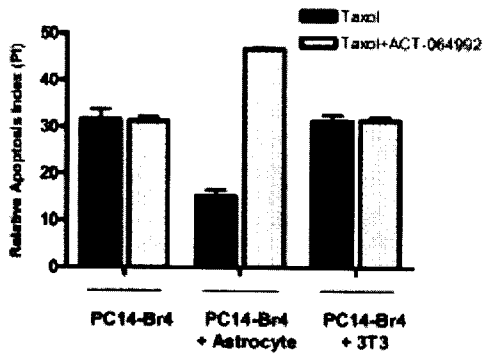

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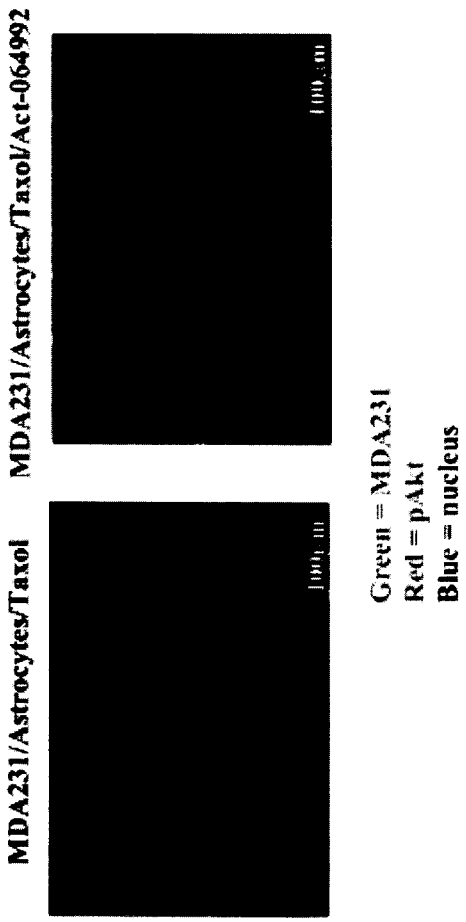
FIGURE 17

Immunohistochemical Analysis



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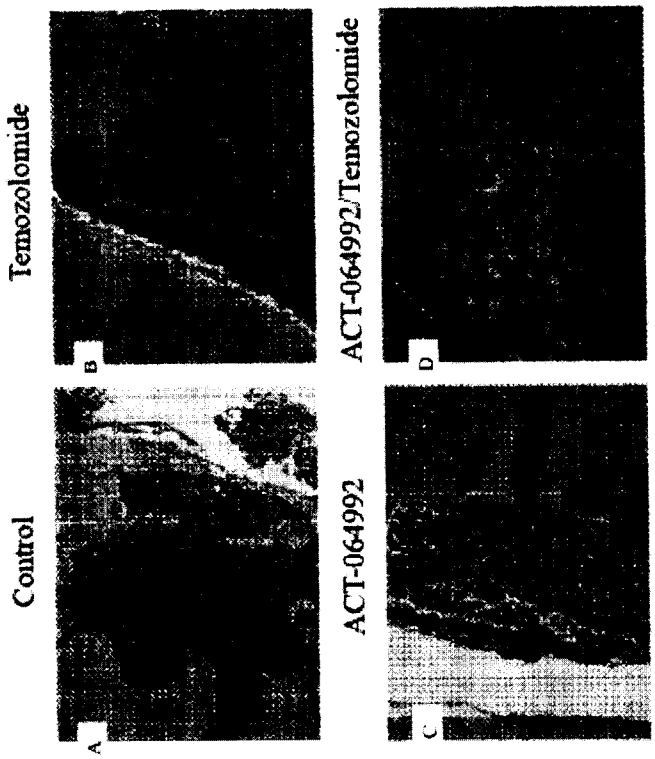

FIGURE 18 A-D

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FIGURE 1SE

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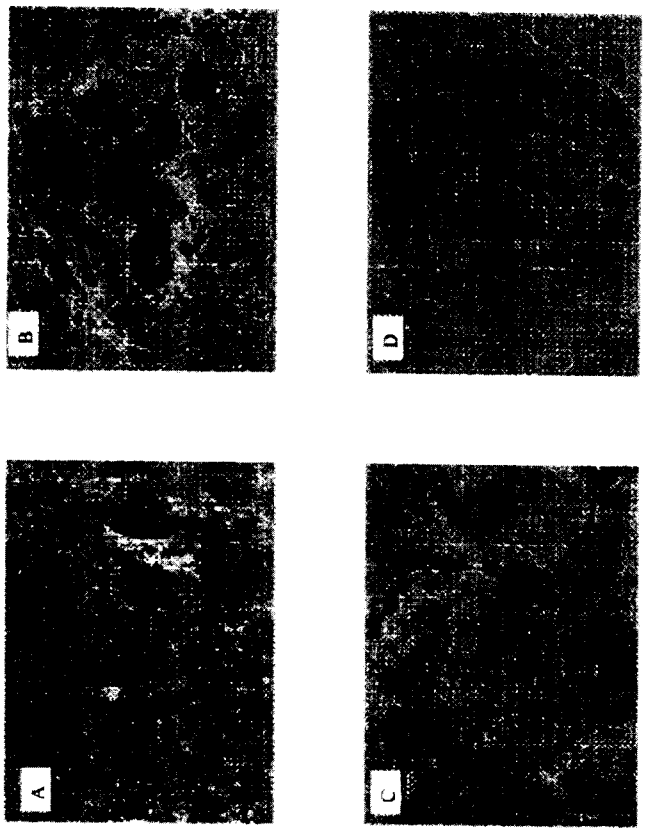

FIGURE 19

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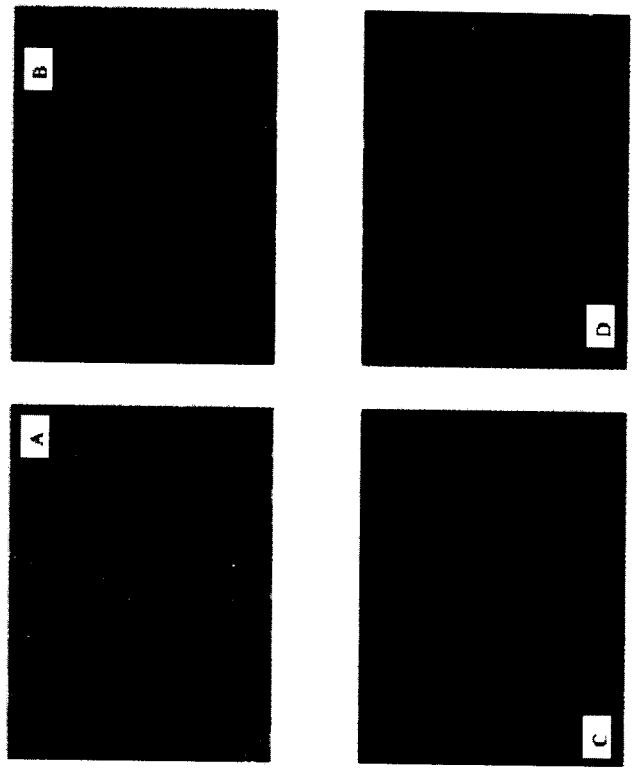

FIGURE 20

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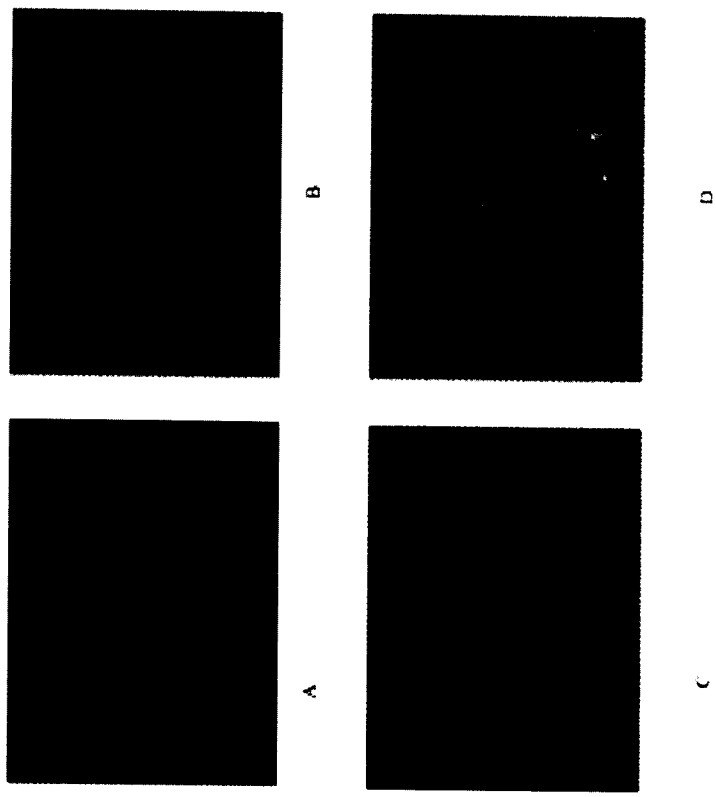

FIGURE 21

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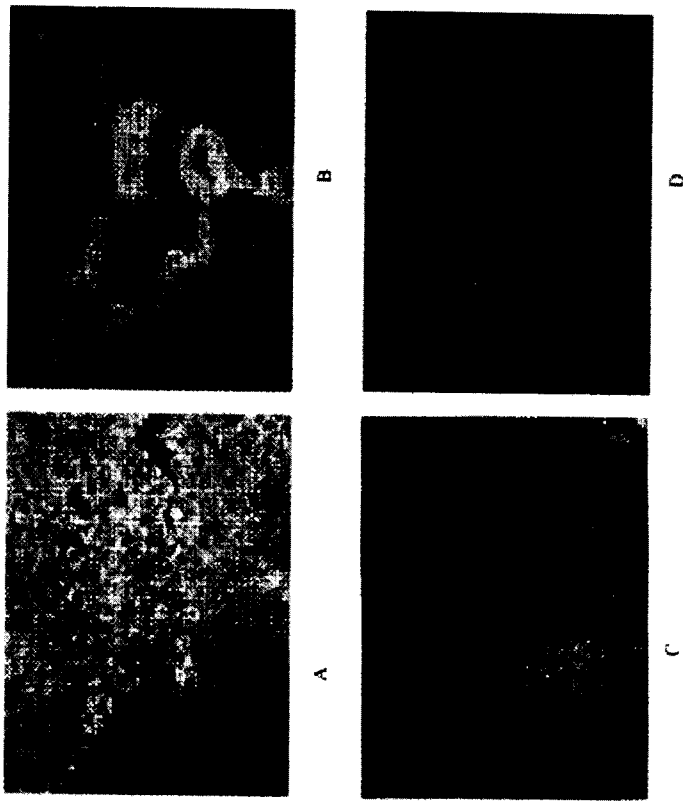

FIGURE 22

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Background

Brain metastasis is one of the most difficult challenges facing oncology. Metastatic tumors are resistant to most chemotherapy agents. The treatments for brain metastasis are primarily whole brain and focused radiotherapy, with surgical resection of tumors in a minority of cases. Most chemotherapy regimens involve 2-3 agents such as cisplatin, cyclophosphamide, etoposide, teniposide, mitomycin, irinotecan, vinorelbine, etoposide, ifosfamide, temozolomide and fluorouracil (5-FU). These are administered in combination with radiotherapy. The effect of these chemotherapies on prolonging survival is generally less than a year. A fairly new chemotherapy for brain tumors is temozolomide used with whole-brain irradiation. Results are preliminary but temozolomide appears to have some limited effect on the response rate compared to radiation alone and appears to have some clinical activity in combination with radiation in phase II trials.

Despite intense efforts, the limited medical options available for brain metastasis have remained poor and too often more palliative than therapeutically effective. This state of affairs has been long recognized but, to date, significant advances have not materialized. Consequently, there is a great and present medical need for new therapeutic approaches and pharmaceuticals effective at treating brain metastasis.

The disclosure below discusses endothelin receptor antagonists in relation to brain metastasis. Endothelin-1 (hereafter "ET-1"), a vasoactive peptide, is produced primarily in endothelial, vascular smooth muscle, and epithelial cells. ET-1 exerts its physiological effect via two high-affinity G-protein-coupled receptors, the endothelin-A (hereafter "ET_A") and the endothelin-B (hereafter "ET_B") receptors. Endothelin receptor antagonists (ERAs) are a well established class of compounds capable of inhibiting these endothelin receptors (hereafter "ETRs"). Within this class are subclasses of antagonists specific to ET_A or ET_B and a subclass effective against both (dual specificity). One member of the dual specificity subclass, bosentan, is currently approved for use in treating pulmonary arterial hypertension.

Certain ERAs have been investigated for use in cancer therapy. [Nelson JB, et al., Phase 3, randomized, controlled trial of atrasentan in patients with nonmetastatic, hormone-refractory prostate cancer. Cancer, 2008 Nov 1;113(9):2376-8.; Chiappori AA, et al. Phase I/II study of atrasentan, an ET_A receptor antagonist, in combination with paclitaxel and carboplatin as

first-line therapy in advanced non-small cell lung cancer. Clin Cancer Res, 2008 Mar 1;14(5):1464-9.] These studies have largely excluded patients with active brain metastasis. *Ibid.* This exclusion is done on the general view that existing brain metastases will not respond to treatment and, thus, morbidity and symptoms due to these metastases would mask the effects of the test treatment on the primary tumor. [Carden CP, et al., Eligibility of patients with brain metastases for phase I trials: time for a rethink? *The Lancet Oncology*, Vol 9, Issue 10, Pages 1012-1017, October 2008 doi:10.1016/S1470-2045(08)70257-2.] This standard clinical trial design strategy serves to emphasize the general expectation that therapies effective against primary tumors and even non-brain metastasis tumors will fail to effect brain metastasis tumors.

Brief Description of the Drawings

FIGURE 1: *In vitro* culture of MDA-MB-231 breast cancer cells (T) and murine astrocytes (A) were evaluated by scanning electron microscopy. Direct contact between the astrocytes (extending pods-feet) and tumor cells is evident;

FIGURE 2: The astrocyte-metastatic cancer cell co-cultures showed dye transfer between co-cultured cells;

FIGURE 3: Culturing of human MDA-MB-231 breast cancer cells or human PC14Br4 lung cancer cells with astrocytes (but not 3T3 fibroblasts) reduced the relative apoptotic index (increased resistance) of tumor cells incubated for 72 hours with paclitaxel (5 ng/ml) by 58.3 + 8.9% (mean ± S.D., $P<0.01$) and 61.8 ± 6.7% (mean ± S.D., $P<0.05$), respectively (the apoptotic index was compared by the Student's *t* test);

FIGURE 4: Human lung cancer PC14Br4 cells were cultured alone or with astrocytes (direct cell to cell contact) in medium containing P-glycoprotein-associated Adriamycin (200 ng/ml), paclitaxel (5 ng/ml), vinblastine (3 ng/ml), vincristine (8 ng/ml), and P-glycoprotein-dissociated 5-FU (500 ng/ml) or cisplatinum (2.4 μg/ml);

FIGURE 5: Astrocyte-mediated protection of brain metastasis cells from cytotoxic chemotherapy-induced cell death does not last longer than 72 hours after direct astrocyte-brain metastasis cell contact is lost;

FIGURE 6: Gene transcription profiling conditions distinguished between murine and human mRNA;

FIGURE 7: In the MDA-MB-231 cells, 1069 genes, and in the PC14Br4 cells, 594 genes were differentially expressed. A two-gene list comparison revealed increased expression of