hypoxia;

Figure 10 is a histogram showing the dose-dependent effects of ranolazine on the lateral motility of MDA-MB-231 cells under normoxia and hypoxia;

Figure 11 is a histogram showing the dose-dependent effect of ranolazine on the transverse migration of MDA-MB-231 cells under normoxia and hypoxia;

Figure 12 is a histogram showing the dose-dependent effect of ranolazine on the invasiveness of MDA-MB-231 cells under normoxia and hypoxia;

Figure 13 is a series of histograms showing the dose-dependent effect of ranolazine on the invasiveness of MDA-MB-231 cells that have been pre-treated with ranolazine for different durations under hypoxia; view (a) is a histogram showing the effect of 5 μM ranolazine for cells treated only for 24 hours during the assay (i.e., no pre-treatment); view (b) is a histogram showing the effect of 5 μM ranolazine for cells pre-treated with the drug for 72 hours; view (c) is a histogram showing the effect of 25 μM ranolazine for cells treated only for 24 hours during the assay (i.e., no pre-treatment); view (d) is a histogram showing the effect of $\geq$25 μM ranolazine for cells pre-treated with the drug for 48 hours, and view (e) is a histogram showing the effect of $\geq$25 μM ranolazine for cells pre-treated with the drug for 72 hours;

In the first phase 107 following time 105, metastatic cells detach themselves from the tumour. Thereafter, in phase 108, they invade and move through surrounding tissue in the same organ towards the circulation system, in particular the vascular and/or the lymphatic system. In phase 109, the metastatic cells enter the circulation system which may then carry them to other organs in the body, at which they may cause the formation of secondary tumours.

The above phases are pictorially represented in Figure 2 in which reference number 200 represents a portion of an organ such as a breast or a prostate. Healthy cells 201 of the breast or prostate are shown as supported on a basement membrane 202 and surrounding a cancerous tumour 203, which is assumed to have gone through the genesis phase 103 and into the proliferation phase 104.

Certain cells 204 of the cancerous tumour ~~202~~ 203 are shown as detaching from the tumour 203 and passing through a degraded region 202a of the basement membrane 202 into adjacent region 205 of the organ containing the tumour 203, which region may comprise mainly collagen fibres. Cancer cells 206, which have become detached from the tumour and have passed through the basement membrane 202, are shown passing through the region 205 towards a blood vessel 207. A cancerous cell 208 is shown migrating through the wall of the blood vessel 207 into the bloodstream 209.

Cells 210, which have already entered the bloodstream, are shown as being

pores 706 extending therethrough. A layer 707 of Matrigel™ is shown coating the filter membrane 705.

Cells 708 were plated at a density of 2 x 10^4 /ml onto the Matrigel™ layer 707 in 24-well plates (Becton-Dickinson) according to the manufacturers' instructions. 50 µl Matrigel™ was seeded at a 1:7 dilution (10 mg/ml stock) onto the inserts and left overnight. Prior to seeding with the cells the Matrigel™ was rehydrated using medium with no additions. This medium was removed prior to seeding the cells.

Cells were plated in a 1-5 % FBS chemotactic gradient overnight (12 hours). The nutrient concentration in the medium 709 in the upper section 703 of the chamber was less than the concentration of nutrient in the medium 710 in the lower section 704 to induced movement of the cells through the Matrigel™ layer 707 and through the pores 706 to the underside of the filter membrane 705. At the end of each assay, non-invaded/non-migrated cells were removed from the upper surface of the insert 702 with two different swabs.

The number of cells invading to the underside of the insert 702 was determined using crystal violet staining. Invaded cells were fixed for 15 minutes with ice-cold methanol. Then 0.5 % crystal violet (in 25 % methanol) was added for 15 minutes. The inserts were swabbed again and then washed in water and allowed to dry. Cells were then counted using twelve separate fields of view per insert (x200

normoxia experiments, the invasiveness of the cells was measured after treating them with no drug (control) and ranolazine at concentrations of 10μM, 20μM, 50μM and 300μM. The results are shown in Figure 12 as blocks 1201, 1203, 1204, 1205 and 1206, respectively. Treatment with ranolazine reduced the ~~migration~~ invasiveness of the cells at concentrations of 10μM, 20μM and 300μM, but the effect of ranolazine at a concentration of 50 μM was not statistically significant.

In the hypoxia experiments, the invasiveness~~migration~~ of the cells was measured after treating them with no drug (control) and ranolazine at concentrations of 5μM, 10μM, 20μM, 50μM and 300μM. The results are shown in Figure 12 as blocks 1207, 1209, 1210, 1211, 1212 and 1213, respectively. Treatment with ranolazine reduced the invasiveness~~migration~~ of the cells at concentrations of 20μM, 50μM and 300μM, but the effect of ranolazine at concentrations of 5 μM and 10 μM was not statistically significant.

Data were collected from n = 3 independent experiments for each condition and are presented as means ± SEM. (*) indicates significance at $P < 0.05$; (**) indicates significance at $P < 0.01$.

Example 6 - Effects of ranolazine on the invasiveness of MDA-MB-231 cells under normoxia and hypoxia with pre-treatment of the cells

As the reduction in invasiveness at low concentrations of ranolazine under hypoxia was not statistically significant, a modified version of the invasiveness

invading over the following 24 hours prior to measurement. Cells pre-treated with 25 μM ranolazine for 24 hours before commencing the 24 hour long assay were significantly less invasive than untreated cells.

In Figure 13(e), block 1309 represents the invasiveness result obtained under control conditions for cells maintained for 48 hours without drug and invading over the following 24 hours prior to measurement. Block 1310 represents the invasiveness result for cells pre-treated for 48 hours with 25 μM ranolazine and invading over the following 24 hours prior to measurement. Cells pre-treated with 25 μM ranolazine for 48 hours before commencing the 24 hour long assay were again significantly less invasive than untreated cells.

As above, the number of "cells invading" in the histograms of Figures 13(c) to (e) denotes the average of cell number counted in 24 randomly chosen fields of view from two separate experiments.

At both the concentrations tested (5 μM and 25 μmol_{-}), pre-treatment of the cells with ranolazine led to a statistically significant reduction in their invasiveness. Such pre-treatment of the cells with the drug in an *in vitro* test is considered to be representative of *in vivo* treatment, where the patient receives a continual therapeutic dose of the drug.

Example 7 - Effects of ranolazine on the growth of MDA-MB-231 cells under

cell number at commencement.

After 24 hours, the number of cells at each concentration of ranolazine was counted again. The results are shown in Figure 14 as blocks 1405, 1406, 1407 and 1408. There was no significant difference between the number of cells in control sample (block 1405) and the numbers of cells in the samples treated with ranolazine at concentrations of 1μM, 10μM and 100μM (blocks 1406, 1407 and 1408, respectively).

After a further 24 hours (48 hours in total), the number of cells at each concentration of ranolazine was counted again. The results are shown in Figure 14 as blocks 1409, 1410, 1411 and 4012. There was no statistically significant difference between the number of cells in control sample (block 1409) and the numbers of cells in the samples treated with ranolazine at concentrations of 1μM, 10μM and 100μM (blocks 1410, 1411 and 4012, respectively).

~~Similarly, in the hypoxia experiments, the motility of the cells was measured after treating them with no drug (control) and ranolazine at concentrations of 1μM, 10μM and 100μM. The results are shown in Figure 10 as blocks 1005, 1006, 1007 and 1008, respectively. Increasing the concentration of ranolazine reduced the lateral motility of the cells, with the result that each concentration of ranolazine being statistically significant.~~

opening of the VGSC. The TTX measurement is a positive control confirming that sodium channel activity is indeed contributing (potentiating) the metastatic cell behaviour under investigation. It should be noted that, at the concentrations of TTX used here (1 μM), blocking of the VGSC is not total. Hence, particularly at the higher concentrations tested, the drug (ranolazine) appears to be more potent than the toxin (TTX).

Block 1706 is the result obtained for the control sample (without drug) for Mat-LyLu cells under conditions of hypoxia. From a comparison of blocks 1701 and 1706, it does not appear that hypoxia affected the invasiveness of the cells. ~~I~~

Rat prostate cancer cells from the Mat-LyLu cell line were treated with different concentrations of ranolazine under normoxia, and hypoxia. In the normoxia experiments, the invasiveness of the cells was measured after treating them with no drug (control) and ranolazine at concentrations of 20μM, 50μM and 300μM. The results are shown in Figure 17 as blocks 1701, 1703, 1704 and 1705, respectively. Treatment with ranolazine reduced the invasiveness~~migration~~ of the cells in a statistically significant way at each of the tested concentrations of ranolazine.

In the hypoxia experiments, the invasiveness~~migration~~ of the cells was measured after treating them with no drug (control) and ranolazine at concentrations of 20μM, -50μM and 300μM. The results are shown in Figure 17 as blocks 1706, 1708, 1709 and 1710, respectively. Treatment with ranolazine reduced the

invasiveness~~migration~~ of the cells in a statistically significant way at each of the tested concentrations of ranolazine.

Data were obtained from $n \geq 3$ independent experiments for each condition and are presented as means ± SEM. (*) indicates significance at $P < 0.05$ compared to normoxia control; (*) indicates significance at $P < 0.05$ compared to both normoxia and hypoxia control.

Example 11 - Effects of ranolazine on growth of Mat-LyLu cells

The growth of the cells was measured using the technique described above under the heading "Cell growth (proliferation) assay ".

Referring to Figure 18, the vertical axis represents the total cell number in a plated sample, with a reference point for normal growth being represented by the control sample (block~~s~~ 1801) of Mat-LyLu cells under normoxia and without drug normalised to 100%. Across the horizontal axis, the set of results for experiments conducted under normoxia is plotted on the left-hand side and the ~~search~~ set of results for experiments conducted under hypoxia is plotted on the right-hand side. For each set of results, the concentration of ranolazine used increases from left to right. Note that the term "growth" includes cell proliferation and cell death.

~~Rack~~ Rat prostate cancer cells from the Mat-LyLu cell line were treated with different concentrations of ranolazine under normoxia, and hypoxia. In the normoxia experiments, the number of cells was measured after treating them for 24 hours

normoxia and hypoxia

Cells were treated with different concentrations of riluzole at [1 μM], [10 μM] and [100 μM] for 48 hours. The results obtained are shown in Figure 25. Riluzole at concentrations of 10 μM and 100 μM decreased the cell viability. Data were collected from n = 3 independent experiments for each condition and are presented as means ± SEM. (*) indicates significant level at $P < 0.05$.

The same concentrations of ~~ranolazine~~ riluzole under hypoxia also did not affect the growth of the MDA-MB-231 cells (results not shown in Figure 25).

Example 20 - Effects of riluzole on Matrigel™ invasion of Mat-LyLu cells under normoxia and hypoxia (no pre--treatment)

The invasiveness of the cells was measured using the technique described above and illustrated in Figure 7. Referring to Figure 26, block 2601 shows the invasion index for the control sample of Mat-LyLu cells under normoxia and without drug, normalised to 100%. Block 2604 is the corresponding control sample (without drug) for Mat-LyLu cells under conditions of hypoxia. From a comparison of blocks 2601 and 2604 it can be seen that hypoxia increased invasion in a statistically significant way.

Riluzole at a concentration of 1 μM significantly inhibited invasiveness under normoxia and hypoxia even without pre--treatment. Data were obtained from $n \geq 3$ independent experiments for each condition and are presented as means ± SEM.

CLAIMS:

1. Use of a substance or composition which has the effect of at least reducing the persistent part of the voltage gated sodium channel (VGSC) current without eliminating the transient part in the manufacture of a medicament for reducing or preventing metastatic behaviour of tumour cells in VGSC-expressing cancer.

2. Use according to claim 1 wherein the medicament reduces or prevents metastatic behaviour without killing the cells.

3. Use according to claim 1 or claim 2 wherein the medicament reduces or prevents metastatic behaviour without substantially affecting proliferation of the cells.

4. Use according to any one of claims 1 to 3 wherein the medicament reduces or prevents at least one of the following metastatic behaviours:

(i) lowering of adhesiveness, and the medicament increases the adhesiveness of the cells of a tumour;

(ii) motility, and the medicament decreases the motility of cells that have become detached from a tumour;

(iii) invasiveness, and the medicament decreases the ability of cells that have become detached from a tumour to move through basement membrane into surrounding tissue, and

(iv) migration, and the medicament decreases the ability of cells that have become detached from a tumour to migrate from surrounding tissue into the patient's circulatory system.

5. Use according to any one of claims 1 to 4 wherein the medicament is for administration to a patient before cells that have become detached from a tumour migrate into the patient's circulatory system.

6. Use according to any one of claims 1 to 4 wherein the medicament is for administration to a patient before cells that have become detached from a tumour and

that have moved through basement membrane into surrounding tissue move through said surrounding tissue towards the patient's circulatory system.

7. Use according to any one of claims 1 to 4 wherein the medicament is for administration to a patient before cells that have become detached from a tumour move through basement membrane into surrounding tissue.

8. Use according to any one of claims 1 to 4 wherein the medicament is for administration to a patient before cells become detached from a tumour.

9. Use according to any one of claims 1 to 4 wherein the medicament is for administration to a patient before the cancer expresses VGSC.

10. Use according to any one of claims 1 to 9 wherein the medicament is for administration to a patient whose tumour exhibits hypoxic conditions.

11. Use according to any one of claims 1 to 9 wherein the medicament is for reducing or preventing metastatic behaviour in VGSC-expressing breast cancer.

12. Use according to any one of claims 1 to 9 wherein the medicament is for reducing or preventing metastatic behaviour in VGSC-expressing prostate cancer.

13. Use according to any one of claims 1 to 12 wherein the substance or composition comprises ranolazine.

14. Use according to any one of claims 1 to 12 wherein the substance or composition comprises riluzole.

15. Use according to any one of claims 1 to 12, wherein the substance or composition comprises valporate, flecainide, lidocaine, mexiletine or F15845.

16. Use according to any one of claims to 15 wherein the substance or

composition is present in a dosage level corresponding to the range 1 μM to 10 μM.

17. Use of ranolazine in the manufacture of a medicament for reducing or preventing metastatic behaviour in VGSC-expressing breast cancer.

18. Use of ranolazine in the manufacture of a medicament for reducing or preventing metastatic behaviour in VGSC-expressing prostate cancer.

~~1~~19. A substance or composition for use in

(a) a method of reducing metastatic behaviour, or

(b) a method of preventing metastatic behaviour

of tumour cells in a patient suffering from cancer that expresses voltage gated sodium channel (VGSC), the substance or composition being in a dose that at least reduces the persistent part of the VGSC current without eliminating the transient part.

~~2.~~20 A substance or composition for use in a method of reducing or preventing metastatic behaviour according to claim ~~1~~19, without killing the cells.

~~3~~21. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to claim ~~1~~19 or claim ~~2~~20, without substantially affecting proliferation of the cells.

~~4~~22. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~3~~21 wherein the metastatic behaviour is at least one of:

(i) lowering of adhesiveness, and the substance or composition increases the adhesiveness of the cells of a tumour;

(ii) motility, and the substance or composition decreases the motility of cells that have become detached from a tumour;

(iii) invasiveness, and the substance or composition decreases the ability of cells that have become detached from a tumour to move through basement membrane into surrounding tissue, and

(iv) migration, and the substance or composition decreases the ability of cells that have become detached from a tumour to migrate from surrounding tissue into the patient's circulatory system.

~~5~~23. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to 4~~22~~ for administration to a patient before cells that have become detached from a tumour migrate into the patient's circulatory system.

~~6~~24. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~4~~22 for administration to a patient before cells that have become detached from a tumour and that have moved through basement membrane into surrounding tissue move through said surrounding tissue towards the patient's circulatory system.

~~7~~25. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~4~~22 for administration to a patient before cells that have become detached from a tumour move through basement membrane into surrounding tissue.

~~8~~26. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~4~~22 for administration to a patient before cells become detached from a tumour.

~~9~~27. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~4~~22 for administration to a patient before the cancer expresses VGSC.

~~10~~28. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~9~~27 for administration to a patient whose tumour exhibits hypoxic conditions.

~~11~~29. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~ 19 to ~~10~~28 wherein the cancer is breast cancer.

~~12~~30. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~10~~28 wherein the cancer is prostate cancer.

~~13~~31. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~12~~30 wherein the substance is ranolazine.

~~14~~32. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~1~~19 to ~~12~~30 wherein the substance is riluzole.

33. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims 19 to 30 wherein the substance or composition comprises valporate, flecainide, lidocaine, mexiletine or F15845.

~~15~~34. A substance or composition for use in a method of reducing or preventing metastatic behaviour according to any one of claims ~~13~~31 ~~or 14~~ to 33, wherein the substance or composition is present in a dosage level corresponding to the range 1 μM~~mol of~~ to 10 μM~~mol~~.

~~16~~35. Ranolazine for use in a method of reducing or preventing metastatic behaviour in VGSC-expressing breast cancer.

36. Ranolazine for use in a method of reducing or preventing metastatic behaviour in VGSC-expressing prostate cancer.