CSCs are thought to be particularly resistant to existing anti-cancer drugs. Even though drug treatment may kill the vast majority of cells in the tumour, a single surviving CSC can therefore lead to a relapse of the disease. Recent evidence suggests an overlap between EMT and CSC phenotypes, suggesting that EMT may also play a role in recurrence of cancer after chemotherapy and the development of drug-resistant tumours.

Robust biomarkers for the EMT phenotype would be useful in identifying patients at particular risk of developing metastatic or drug-resistant cancer, while novel drugs that target cells that have undergone EMT will reduce metastasis and relapse following conventional therapy.

EMT activators (e.g. the transcription factor Slug) increase Akt1 activity/expression. It is also known that Akt1 activation (for example of the myristylated variant MyrAkt1) induces EMT activators (e.g. the transcriptional repressor, Snail; Oncogene. 2007 Nov 22;**26**(53):7445-56. Epub 2007 Jun 1) and also causes biomarker switching from epithelial to mesenchymal.

Summary ~~of the Invention~~

In one aspect, there is provided a method of identifying a subject undergoing epithelial-to-mesenchymal transition (EMT), the method comprising determining the level of Akt3 expression or activity in a sample derived from the subject relative to a control sample, an increase in expression or activity of Akt3 indicating the occurrence of EMT.

In another aspect, there is provided a prognostic method for either one of: (i) determining whether a subject will be susceptible to treatment with an ~~Akt3 or~~ Ax1 inhibitor; or (ii) determining whether a subject is particularly likely to suffer from metastatic cancer; said method comprising detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in said subject by assessing Akt3 activity or expression in a sample derived from the subject relative to a control sample.

In another aspect, there is provided a method of identifying Axl activity, the method comprising determining the level of Akt3 expression or activity in a sample derived from a subject relative to a control sample, increased expression or activity of Akt3 correlating with Axl activity.

In another aspect, there is provided a method of selecting patients, preferably human patients, for treatment of an Akt3-related condition, the method comprising identifying patients having indications of the occurrence of EMT and selecting thus identified patients for treatment, wherein patients having indications of the occurrence of EMT are identified by a method comprising determining the level of Akt3 expression or activity in a sample derived from the patient, an increase in expression or activity of Akt3 indicating the occurrence of EMT; and wherein the treatment comprises administering an Axl inhibitor.~~, optionally wherein the Akt3-related condition is cancer.~~

In another aspect, there is provided the use of an EMT inhibitor ~~for~~in the manufacture of a medicament ~~in~~for the treatment of an Akt3-related condition, optionally wherein the condition is cancer.

In another aspect, there is provided the use of an Akt3 inhibitor ~~for~~in the manufacture of a medicament ~~in~~for the ~~inhibition~~treatment of an EMT-related disease.

In another aspect, there is provided the use of a compound capable of inhibiting Akt3 activity ~~for~~in the manufacture of a medicament ~~in~~for the prevention, inhibition, or treatment of drug resistance in a subject having cancer, the method comprising contacting the subject with a compound capable of inhibiting Akt3 activity.

In another aspect, there is provided the use of an ~~Akt3~~EMT inhibitor or pharmaceutical compound selected as, or derived from, a candidate compound obtained by a method disclosed herein, for the manufacture of a medicament for use in a method of treating a subject having an Akt3-related condition.

In another aspect, there is provided the use of a compound capable of inhibiting EMT ~~for~~in the manufacture of a medicament for use in a method of treating an Akt3-related condition in a subject.

In another aspect, there is provided the use of a compound capable of inhibiting Akt3 activity ~~for~~in the manufacture of a medicament for use in a method of inhibiting Cancer Stem cells in a subject.

In another aspect, there is provided the use of a compound capable of inhibiting Akt3 activity ~~for~~in the manufacture of a medicament for use in a method of preventing or inhibiting drug resistance in a subject having cancer.

In another aspect, there is provided a method of selecting a candidate pharmaceutical compound useful: (i) for the prevention, inhibition or treatment of an Akt3-related condition; (ii) in the treatment of metastatic or drug resistant cancer; or (iii) in the prevention or inhibition of EMT; the method comprising providing a group of candidate pharmaceutical compounds for testing, testing the effect of candidate pharmaceutical compounds on occurrence of EMT in a test system, and selecting a candidate pharmaceutical compound on the basis of inhibiting Akt3 activity.

In another aspect, there is provided a method of selecting a candidate pharmaceutical compound useful in the prevention, inhibition or treatment of an Akt3-related condition, the method comprising: (i) selectively reducing expression of Akt3 in a test cell, contacting the test cell with the candidate pharmaceutical compound and determining the effect of the candidate pharmaceutical compound on the occurrence of EMT; or (ii) selectively reducing expression of Akt3 in an in vitro test system to a low level contacting the test system with a candidate pharmaceutical compound, and selecting candidate pharmaceutical compounds which inhibit the occurrence of EMT.

Claims

1. A method of identifying a subject undergoing epithelial-to-mesenchymal transition (EMT), the method comprising determining the level of Akt3 expression or activity in a sample derived from the subject relative to a control sample, an increase in expression or activity of Akt3 indicating the occurrence of EMT.

2. A method according to claim 1, wherein the occurrence of EMT indicated by an increased level of Akt3 expression or activity indicates an increased risk of the subject of developing metastatic or drug-resistant cancer.

3. A method according to claim 1, wherein the occurrence of EMT indicated by an increased expression or activity of Akt3 indicates the existence of a Cancer Stem Cell (CSC).

4. A prognostic method for either one of:

(i) determining whether a subject will be susceptible to treatment with an Axl inhibitor; or

(ii) determining whether a subject is particularly likely to suffer from metastatic cancer;

said method comprising detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in said subject by assessing Akt3 activity or expression in a sample derived from the subject relative to a control sample.

5. A method according to claim 4, wherein an increase in Akt3 activity or expression relative to a control sample is indicative of susceptibility to treatment with an agent capable of inhibiting or reversing EMT, optionally wherein the agent is an Akt3 inhibitor or an Axl inhibitor.

6. A method of identifying Axl activity, the method comprising determining the level of Akt3 expression or activity in a sample derived from a subject relative to a control sample, increased expression or activity of Akt3 correlating with Axl activity.

7. A method according to any one of claims 1 to 6 in which the subject is mammalian, optionally wherein the subject is human.

8. A method according to any one of claims 1 to 7, further comprising assessing the level of expression or activity of Akt2 in a sample derived from the subject, wherein a decreased level of Akt2 expression or activity indicates:
(i) the subject has an Akt3-related condition;
(ii) an increased risk of developing metastic or drug-resistant cancer;
(iii) the existence of a cancer stem cell; and/or
(iv) the occurrence of EMT.

9. A method according to any one of claims 1 to 8 in which expression of Akt3 and not Akt1 and/or Akt2 is determined.

10. A method according to any one of claims 1 to 9, wherein the level of expression of Akt3 is assessed by determining:

(i) the copy number of the gene encoding Akt3 relative to a control sample, wherein an increase in the copy number indicates an increased level of expression of Akt3; and/or

(ii) the level of Akt3 protein or mRNA.

11. A method according to any one of claims 1 to 10, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates active Akt3, optionally wherein Akt3 phosphorylation is determined at Serine 472; and/or

(ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.

12. A method of selecting patients, preferably human patients, for treatment of an Akt3-related condition, the method comprising identifying patients having indications of the occurrence of EMT and selecting thus identified patients for treatment,

wherein patients having indications of the occurrence of EMT are identified by a method comprising determining the level of Akt3 expression or activity in a sample

derived from the patient, an increase in expression or activity of Akt3 indicating the occurrence of EMT;

and wherein the treatment comprises administering an Axl inhibitor.

13. A method of selecting patients according to claim 12, wherein the Akt3-related condition is cancer.

14. A method according to any one of claims 2, 4 to 5, or 7 to 13, wherein the cancer or Akt3-related condition is a cancer selected from breast, melanoma, prostate, ovarian, colorectal, lung or glioma cancer.

15. Use of an EMT inhibitor in the manufacture of a medicament for the treatment of an Akt3-related condition, optionally wherein the condition is cancer.

16. Use of an Akt3 inhibitor in the manufacture of a medicament for the treatment of an EMT-related disease.

17. Use of a compound capable of inhibiting Akt3 activity in the manufacture of a medicament for the prevention, inhibition, or treatment of drug resistance in a subject having cancer, the method comprising contacting the subject with a compound capable of inhibiting Akt3 activity.

18. The use according to any one of claims 16 to 17, wherein the medicament is in combination with another therapeutic agent, wherein the other therapeutic agent is a cancer treatment selected from:

(i) alkylating agents, including alkyl sulfonates such as busulfan;

(ii) nitrogen mustards such as chlorambucil, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, and uramustine;

(iii) ethyleneimine derivatives such as thiotepa;

(iv) nitrosoureas such as carmustine, lomustine, and streptozocin;

(v) triazenes such as dacarbazine, procarbazine, and temozolamide;

(vi) platinum compounds such as cisplatin, carboplatin, oxaliplatin, satraplatin, and picoplatin onnaplatin, tetraplatin, sprioplatin, iproplatin,

chloro(diethylenediamino)-platinum (II) chloride, dichloro(ethylenediamino)-platinum (II), diamino(2-ethylmalonato)platinum (II), (1 ,2-diaminocyclohexane)malonatoplatinum (II), (4-carboxyphthalo)-(1,2-diaminocyclohexane)platinum (II), (1 ,2-diaminocyclohexane)-(isocitrato)platinum (II), and (1 ,2-diaminocyclohexane)-cis-(pyruvato)platinum (II);

(vii) antimetabolites, including antifolates such as methotrexate, permetrexed, raltitrexed, and trimetrexate;

(viii) pyrimidine analogues such as azacitidine, capecitabine, cytarabine, edatrexate, floxuridine, fluorouracil, gemcitabine, and troxacitabine;

(ix) purine analogues such as cladribine, chlorodeoxyadenosine, clofarabine, fludarabine, mercaptopurine, pentostatin, and thioguanine;

(x) natural products, including antitumor antibiotics such as bleomycin, dactinomycin, mithramycin, mitomycin, mitoxantrone, porfiromycin;

(xi) anthracyclines such as daunorubicin, doxorubicin, epirubicin, idarubicin, and valrubicin;

(xii) mitotic inhibitors such as the vinca alkaloids vinblastine, vinvesir, vincristine, vindesine, and vinorelbine;

(xiii) enzymes such as L-asparaginase and PEG-L-asparaginase;

(xiv) microtubule polymer stabilizers such as the taxanes paclitaxel and docetaxel;

(xv) topoisomerase I inhibitors such as the camptothecins irinotecan and topotecan;

(xvi) topoisomerase II inhibitors such as podophyllotoxin, amsacrine, etoposide, teniposide, losoxantrone and actinomycin;

(xvii) hormones and hormone antagonists, including

- androgens such as fluoxymesterone and testolactone;
- antiandrogens such as bicalutamide, cyproterone, flutamide, and nilutamide;
- corticosteroids such as dexamethasone and prednisone;
- aromatase inhibitors such as aminoglutethimide, anastrozole, exemestane, formestane, and letrozole;
- estrogens such as diethylstilbestrol;

- antiestrogens such as fulvestrant, raloxifene, tamoxifen, and toremifine;
- luteinising hormone-releasing hormone (LHRH) agonists and antagonists such as abarelix, buserelin, goserelin, leuprolide, histrelin, desorelin, nafarelin acetate and triptorelin;
- progestins such as medroxyprogesterone acetate and megestrol acetate; and
- thyroid hormones such as levothyroxine and liothyronine;

(xviii) PKB pathway inhibitors, including perifosine, enzastaurin hydrochloride, and triciribine;

(xix) P13K inhibitors such as semaphore and SF1126, and MTOR inhibitors such as rapamycin and analogues;

(xx) CDK inhibitors, including seliciclib, alvocidib, and 7-hydroxystaurosporine;

(xxi) COX-2 inhibitors, including celecoxib;

(xxii) HDAC inhibitiors, including trichostatin A, suberoylanilide hydroxamic acid, and chlamydocin;

(xxiii) DNA methylase inhibitors, including temozolomide, and miscellaneous agents, including altretamine, arsenic trioxide, thalidomide, lenalidomide, gallium nitrate, levamisole, mitotane, hydroxyurea, octreotide, procarbazine, suramin, photodynamic compounds such as methoxsalen and sodium porfimer;

(xxiv) proteasome inhibitors such as bortezomib;

(xxv) molecular targeted therapy agents including: functional therapeutic agents, including gene therapy agents and antisense therapy agents;

(xxvi) tyrosine kinase inhibitors such as erlotinib hydrochloride, gefitinib, imatinib mesylate, and semaxanib;

(xxvii) Raf inhibitors such as sorafenib, and gene expression modulators such as the retinoids and rexinoids, for example adapalene, bexarotene, trans-retinoic acid, 9-cis-retinoic acid, and N-(4-hydroxyphenyl)retinamide;

(xxviii) phenotype-directed therapy agents, including monoclonal antibodies such as alemtuzumab, bevacizumab, cetuximab, ibritumomab tiuxetan, rituximab, and trastuzumab, immunotoxins such as gemtuzumab ozogamicin, radioimmunoconjugates such as I-tositumobab;

(xxix) cancer vaccines;

(xxx) biologic therapy agents including: interferons such as interferon-[alpha]2a and interferon-[alpha]2b, and interleukins such as aldesleukin, denileukin diftitox, and oprelvekin;

(xxxi) anticancer therapies involving the use of protective or adjunctive agents, including: cytoprotective agents such as amifostine, and dexrazoxane, phosphonates such as pamidronate and zoledronic acid, and stimulating factors such as epoetin, darbeopetin, filgrastim, PEG-filgrastim, and sargramostim;

(xxxii) Axl inhibitors such as 1-(6,7-dihydro-5H-benzo[6,7]cyclohepta[1,2-c]pyridazin-3-yl)-N3-((7-(S)-pyrrolidin-1-yl)-6,7,8,9-tetrahydro-5H-benzo[7]annulene-2-yl)-1H-1,2,4-triazole-3,5-diamine; and/or

(xxxiii) further combination chemotherapeutic regimens, such as combinations of carboplatin/paclitaxel, capecitabine/docetaxel, fluorauracil/levamisole, fluorauracil/leucovorin, methotrexate/leucovorin, and trastuzumab/paclitaxel, alone or in further combination with carboplatin.

19. A method of selecting a candidate pharmaceutical compound useful:

(i) for the prevention, inhibition or treatment of an Akt3-related condition;

(ii) in the treatment of metastatic or drug resistant cancer; or

(iii) in the prevention or inhibition of EMT;

the method comprising providing a group of candidate pharmaceutical compounds for testing, testing the effect of candidate pharmaceutical compounds on occurrence of EMT in a test system, and selecting a candidate pharmaceutical compound on the basis of inhibiting Akt3 activity.

20. A method of selecting a candidate pharmaceutical compound useful in the prevention, inhibition or treatment of an Akt3-related condition, the method comprising:

(i) selectively reducing expression of Akt3 in a test cell, contacting the test cell with the candidate pharmaceutical compound and determining the effect of the candidate pharmaceutical compound on the occurrence of EMT; or

(ii) selectively reducing expression of Akt3 in an in vitro test system to a low level, contacting the test system with a candidate pharmaceutical compound, and selecting candidate pharmaceutical compounds which inhibit the occurrence of EMT.

21. A method according to claim 19 or 20 in which candidate pharmaceutical compounds which substantially or completely inhibit the occurrence of EMT are selected.

22. A method of selecting candidate pharmaceutical compounds according to claim 20 or 21 in which a reduction in EMT is indicated by inhibition of Akt3 activity.

23. A method according to claim 22 in which the expression of Akt3 in cells in the test system is reduced by 90%, 80%, 70%, 60%, 50%, 40%, 30%, or 20%, optionally wherein the expression of Akt3 is reduced so as to not cause inhibition of EMT.

24. A method according to any one of claims 19 to 23 in which the expression of Akt3 is selectively reduced by introducing into cells in the test system a nucleotide which interferes with expression of Akt3.

25. Use of an EMT inhibitor or pharmaceutical compound selected as, or derived from, a candidate compound obtained by a method according to any one of claims 19 to 20 in the manufacture of a medicament for use in a method of treating a subject having an Akt3-related condition.

26. The use according to claim 25, wherein the Akt3-related condition is cancer.

27. The use according to claim 25 or 26, wherein treatment of the subject is adjusted according to detected levels of Akt3 activity in the subject.

28. The use according to any one of claims 25 to 27, wherein the medicament is to be administered with an Axl inhibitor.

29. Use of a compound capable of inhibiting EMT in the manufacture of a medicament for use in a method of treating an Akt3-related condition in a subject.

30. Use of a compound capable of inhibiting Akt3 activity in the manufacture of a medicament for use in a method of inhibiting Cancer Stem cells in a subject.

31. Use of a compound capable of inhibiting Akt3 activity in the manufacture of a medicament for use in a method of preventing or inhibiting drug resistance in a subject having cancer.

32. The use according to any one of claims 25 to 31, wherein the subject is mammalian, optionally wherein the subject is human.

33. The use according to any one of claims 25 to 32, wherein the medicament is to be administered with another cancer therapeutic selected from:

(i) alkylating agents, including alkyl sulfonates such as busulfan;

(ii) nitrogen mustards such as chlorambucil, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, and uramustine;

(iii) ethyleneimine derivatives such as thiotepa;

(iv) nitrosoureas such as carmustine, lomustine, and streptozocin;

(v) triazenes such as dacarbazine, procarbazine, and temozolamide;

(vi) platinum compounds such as cisplatin, carboplatin, oxaliplatin, satraplatin, and picoplatin onnaplatin, tetraplatin, sprioplatin, iproplatin, chloro(diethylenediamino)-platinum (II) chloride, dichloro(ethylenediamino)-platinum (II), diamino(2-ethylmalonato)platinum (II), (1 ,2-diaminocyclohexane)malonatoplatinum (II), (4-carboxyphthalo)-(1,2-diaminocyclohexane)platinum (II), (1 ,2-diaminocyclohexane)-(isocitrato)platinum (II), and (1 ,2-diaminocyclohexane)-cis-(pyruvato)platinum (II);

(vii) antimetabolites, including antifolates such as methotrexate, permetrexed, raltitrexed, and trimetrexate;

(viii) pyrimidine analogues such as azacitidine, capecitabine, cytarabine, edatrexate, floxuridine, fluorouracil, gemcitabine, and troxacitabine;

(ix) purine analogues such as cladribine, chlorodeoxyadenosine, clofarabine, fludarabine, mercaptopurine, pentostatin, and thioguanine;

(x) natural products, including antitumor antibiotics such as bleomycin, dactinomycin, mithramycin, mitomycin, mitoxantrone, porfiromycin;

(xi) anthracyclines such as daunorubicin, doxorubicin, epirubicin, idarubicin, and valrubicin;

(xii) mitotic inhibitors such as the vinca alkaloids vinblastine, vinvesir, vincristine, vindesine, and vinorelbine;

(xiii) enzymes such as L-asparaginase and PEG-L-asparaginase;

(xiv) microtubule polymer stabilizers such as the taxanes paclitaxel and docetaxel;

(xv) topoisomerase I inhibitors such as the camptothecins irinotecan and topotecan;

(xvi) topoisomerase II inhibitors such as podophyllotoxin, amsacrine, etoposide, teniposide, losoxantrone and actinomycin;

(xvii) hormones and hormone antagonists, including

- androgens such as fluoxymesterone and testolactone;
- antiandrogens such as bicalutamide, cyproterone, flutamide, and nilutamide;
- corticosteroids such as dexamethasone and prednisone;
- aromatase inhibitors such as aminoglutethimide, anastrozole, exemestane, formestane, and letrozole;
- estrogens such as diethylstilbestrol;
- antiestrogens such as fulvestrant, raloxifene, tamoxifen, and toremifine;
- luteinising hormone-releasing hormone (LHRH) agonists and antagonists such as abarelix, buserelin, goserelin, leuprolide, histrelin, desorelin, nafarelin acetate and triptorelin;
- progestins such as medroxyprogesterone acetate and megestrol acetate; and
- thyroid hormones such as levothyroxine and liothyronine;

(xviii) PKB pathway inhibitors, including perifosine, enzastaurin hydrochloride, and triciribine;

(xix) P13K inhibitors such as semaphore and SF1126, and MTOR inhibitors such as rapamycin and analogues;

(xx) CDK inhibitors, including seliciclib, alvocidib, and 7-hydroxystaurosporine;

(xxi) COX-2 inhibitors, including celecoxib;

(xxii) HDAC inhibitiors, including trichostatin A, suberoylanilide hydroxamic acid, and chlamydocin;

(xxiii) DNA methylase inhibitors, including temozolomide, and miscellaneous agents, including altretamine, arsenic trioxide, thalidomide, lenalidomide, gallium nitrate, levamisole, mitotane, hydroxyurea, octreotide, procarbazine, suramin, photodynamic compounds such as methoxsalen and sodium porfimer;

(xxiv) proteasome inhibitors such as bortezomib;

(xxv) molecular targeted therapy agents including: functional therapeutic agents, including gene therapy agents, antisense therapy agents;

(xxvi) tyrosine kinase inhibitors such as erlotinib hydrochloride, gefitinib, imatinib mesylate, and semaxanib;

(xxvii) Raf inhibitors such as sorafenib, and gene expression modulators such as the retinoids and rexinoids, for example adapalene, bexarotene, trans-retinoic acid, 9-cis-retinoic acid, and N-(4-hydroxyphenyl)retinamide;

(xxviii) phenotype-directed therapy agents, including monoclonal antibodies such as alemtuzumab, bevacizumab, cetuximab, ibritumomab tiuxetan, rituximab, and trastuzumab, immunotoxins such as gemtuzumab ozogamicin, radioimmunoconjugates such as I-tositumobab;

(xxix) cancer vaccines;

(xxx) biologic therapy agents including: interferons such as interferon-[alpha]2a and interferon-[alpha]2b, and interleukins such as aldesleukin, denileukin diftitox, and oprelvekin;

(xxxi) anticancer therapies involving the use of protective or adjunctive agents, including: cytoprotective agents such as amifostine, and dexrazoxane, phosphonates such as pamidronate and zoledronic acid, and stimulating factors such as epoetin, darbeopetin, filgrastim, PEG-filgrastim, and sargramostim;

(xxxii) Axl inhibitors such as 1-(6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazin-3-yl)-N^3-((7-(*S*)-pyrrolidin-1-yl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-2-yl)-1*H*-1,2,4-triazole-3,5-diamine; and/or

(xxxiii) further combination chemotherapeutic regimens, such as combinations of carboplatin/paclitaxel, capecitabine/docetaxel, fluorauracil/levamisole, fluorauracil/leucovorin, methotrexate/leucovorin, and trastuzumab/paclitaxel, alone or in further combination with carboplatin.

34. A cell line which is sensitive to inhibitors of EMT, the cell line having a level of Akt3 expression that is just insufficient to prevent EMT, optionally wherein the cell line is a human cell line.

35. A method of identifying a compound which inhibits EMT, the method comprising contacting a cell from a cell line according to claim 34 with a test compound and determining inhibition of Akt3 activity in the cell.

36. Use of Akt3 as a biomarker for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a subject, optionally wherein an increase in the expression and/or activation of Akt3 is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

37. Use of Akt3 as a biomarker for detecting the expression and/or activation of Axl, wherein an increase in the expression and/or activation of Akt3 is indicative of an increase in the expression and/or activation of Axl.

38. A method for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a sample, said method comprising

determining the expression level or activation of Akt3 in a sample isolated from a cell, group of cells, an animal model or human as compared to a control sample, wherein an increase in the expression level or activation of Akt3 relative to the control sample is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

39. A method for identifying an agent capable of inhibiting or reversing epithelial-to mesenchymal transition (EMT) , said method comprising administering said agent to an *in vitro* cell or group of cells, or animal model, and monitoring the activation and/or the expression of Akt3, optionally wherein the method further comprises:

(i) administering the agent to a cell, group of cells or an animal model, not a human; and

(ii) measuring Akt3 expression and/or Akt3 activation in samples derived from the treated and the untreated cells or animal model; and

(iii) detecting an increase in the expression and/or activation of Akt3 in the treated sample as compared to the untreated sample as an indication of the ability to inhibit or reverse epithelial-to-mesenchymal transition (EMT).

40. A method according to claim 41, wherein the animal model is not a human.

41. A use or method according to any one of claims 36 to 40 wherein the level of expression of Akt3 is assessed by determining:

(i) the copy number of the gene encoding Akt3 relative to a control sample, wherein an increase in the copy number indicates an increased level of expression of Akt3; and/or

(ii) the level of Akt3 protein or mRNA.

42. A use or method according to any one of claims 36 to 41, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates Akt3 activity, optionally wherein Akt3 phosphorylation is determined at Serine 472; and/or

(ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.

Claims

1. A method of identifying a subject undergoing epithelial-to-mesenchymal transition (EMT), the method comprising determining the level of Akt3 expression or activity in a sample derived from the subject relative to a control sample, an increase in expression or activity of Akt3 indicating the occurrence of EMT.

2. A method according to claim 1, wherein the occurrence of EMT indicated by an increased level of Akt3 expression or activity indicates an increased risk of the subject of developing metastatic or drug-resistant cancer.

3. A method according to claim 1, wherein the occurrence of EMT indicated by an increased expression or activity of Akt3 indicates the existence of a Cancer Stem Cell (CSC).

4. A prognostic method for either one of:

(i) determining whether a subject will be susceptible to treatment with an Akt3 or Ax1 inhibitor; or

(ii) determining whether a subject is particularly likely to suffer from metastatic cancer;

said method comprising detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in said subject.

5. A method according to claim 4, the method comprising assessing Akt3 activity or expression in a sample derived from the subject relative to a control sample.

6. A method according to claim 5, wherein an increase in Akt3 activity or expression relative to a control sample is indicative of susceptibility to treatment with an agent capable of inhibiting or reversing EMT, optionally wherein the agent is an Akt3 inhibitor or an Axl inhibitor.

7. A method of identifying Axl activity, the method comprising determining the level of Akt3 expression or activity in a sample derived from a subject relative to a control sample, increased expression or activity of Akt3 correlating with Axl activity.

8. A method according to any one of claims 1 to 7 in which the subject is mammalian, optionally wherein the subject is human.

9. A method according to any one of claims 1 to 8, further comprising assessing the level of expression or activity of Akt2 in a sample derived from the subject, wherein a decreased level of Akt2 expression or activity indicates:
(i) the subject has an Akt3-related condition;
(ii) an increased risk of developing metastic or drug-resistant cancer;
(iii) the existence of a cancer stem cell; and/or
(iv) the occurrence of EMT.

10. A method according to any one of claims 1 to 9 in which expression of Akt3 and not Akt1 and/or Akt2 is determined.

11. A method according to any one of claims 1 to 10, wherein the level of expression of Akt3 is assessed by determining:

(i) the copy number of the gene encoding Akt3 relative to a control sample, wherein an increase in the copy number indicates an increased level of expression of Akt3; and/or

(ii) the level of Akt3 protein or mRNA.

12. A method according to any one of claims 1 to 11, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates active Akt3, optionally wherein Akt3 phosphorylation is determined at Serine 472; and/or

(ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.

13. A method of selecting patients, preferably human patients, for treatment of an Akt3-related condition, the method comprising identifying patients having indications of the occurrence of EMT and selecting thus identified patients for treatment, optionally wherein the Akt3-related condition is cancer.

14. A method according to claim 13, wherein the patient is identified according to a method of any one of claims 1 to 12.

15. A method according to any one of claims 13 to 14, wherein the treatment comprises administering an agent capable of inhibiting or reversing EMT, optionally wherein the agent comprises an Akt3 inhibitor or an Axl inhibitor.

16. A method according to any one of claims 1 to 15, wherein the cancer or Akt3-related condition is a cancer selected from breast, melanoma, prostate, ovarian, colorectal, lung or glioma cancer.

17. Use of an EMT inhibitor for the manufacture of a medicament in the treatment of an Akt3-related condition, optionally wherein the condition is cancer.

18. Use of an Akt3 inhibitor for the manufacture of a medicament in the inhibition of EMT.

19. Use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament in the prevention, inhibition, or treatment of drug resistance in a subject having cancer, the method comprising contacting the subject with a compound capable of inhibiting Akt3 activity.

20. The use according to any one of claims 18 to 19, wherein the medicament is in combination with another therapeutic agent.

21. A method of selecting a candidate pharmaceutical compound useful:

(i) for the prevention, inhibition or treatment of an Akt3-related condition;

(ii) in the treatment of metastatic or drug resistant cancer; or

(iii) in the prevention or inhibition of EMT;

the method comprising providing a group of candidate pharmaceutical compounds for testing, testing the effect of candidate pharmaceutical compounds on occurrence of EMT in a test system, and selecting a candidate pharmaceutical compound on the basis of inhibiting Akt3 activity.

22. A method of selecting a candidate pharmaceutical compound useful in the prevention, inhibition or treatment of an Akt3-related condition, the method comprising:

(i) selectively reducing expression of Akt3 in a test cell, contacting the test cell with the candidate pharmaceutical compound and determining the effect of the candidate pharmaceutical compound on the occurrence of EMT; or

(ii) selectively reducing expression of Akt3 in an in vitro test system to a low level, contacting the test system with a candidate pharmaceutical compound, and selecting candidate pharmaceutical compounds which inhibit the occurrence of EMT.

23. A method according to claim 21 or 22 in which candidate pharmaceutical compounds which substantially or completely inhibit the occurrence of EMT are selected.

24. A method of selecting candidate pharmaceutical compounds according to claim 22 or 23 in which a reduction in EMT is indicated by inhibition of Akt3 activity.

25. A method according to claim 24 in which the expression of Akt3 in cells in the test system is reduced by 90%, 80%, 70%, 60%, 50%, 40%, 30%, or 20%, optionally wherein the expression of Akt3 is reduced so as to not cause inhibition of EMT.

26. A method according to any one of claims 21 to 25 in which the expression of Akt3 is selectively reduced by introducing into cells in the test system a nucleotide which interferes with expression of Akt3.

27. Use of an Akt3 inhibitor or pharmaceutical compound selected as, or derived from, candidate compound obtained by a method according to any one of claims 21 to 22 for the manufacture of a medicament in a method of treating a subject having an Akt3-related condition

28. The use according to claim 27, wherein the Akt3-related condition is cancer.

29. The use according to claim 27 or 28, wherein the medicament is adjusted according to detected levels of Akt3 activity in the subject.

30. The use according to any one of claims 27 to 29, wherein the medicament is to be administered with an Axl inhibitor.

31. Use of a compound capable of inhibiting EMT for the manufacture of a medicament in a method of treating an Akt3-related condition in a subject.

32. Use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament in a method of inhibiting Cancer Stem cells in a subject.

33. The use according to any one of claims 27 to 32, wherein the medicament is to be administered with another cancer therapeutic.

34. Use of a compound capable of inhibiting Akt3 activity for the manufacture of a medicament in a method of preventing or inhibiting drug resistance in a subject having cancer.

35. The use according to any one of claims 27 to 34, wherein the subject is mammalian, optionally wherein the subject is human.

36. The use according to any one of claims 20, or any one of claims 33 to 35 wherein the other therapeutic agent is a cancer treatment selected from alkylating agents, including alkyl sulfonates such as busulfan, nitrogen mustards such as chlorambucil, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, melphalan, and uramustine, ethyleneimine derivatives such as thiotepa, nitrosoureas such as carmustine, lomustine, and streptozocin, triazenes such as dacarbazine, procarbazine, and temozolamide, platinum compounds such as cisplatin, carboplatin, oxaliplatin, satraplatin, and picoplatin onnaplatin, tetraplatin, sprioplatin, iproplatin, chloro(diethylenediamino)-platinum (II) chloride, dichloro(ethylenediamino)-platinum (II), diamino(2-ethylmalonato)platinum (II), (1 ,2-diaminocyclohexane)malonatoplatinum (II), (4-carboxyphthalo)-(1,2-diaminocyclohexane)platinum (II), (1 ,2-diaminocyclohexane)-(isocitrato)platinum (II), and (1 ,2-diaminocyclohexane)-cis-(pyruvato)platinum (II); antimetabolites, including antifolates such as methotrexate, permetrexed, raltitrexed, and

trimetrexate,pyrimidine analogues such as azacitidine, capecitabine, cytarabine, edatrexate, floxuridine, fluorouracil, gemcitabine, and troxacitabine, and purine analogues such as cladribine, chlorodeoxyadenosine, clofarabine, fludarabine, mercaptopurine, pentostatin, and thioguanine; natural products, including antitumor antibiotics such as bleomycin, dactinomycin, mithramycin, mitomycin, mitoxantrone, porfiromycin, and anthracyclines such as daunorubicin, doxorubicin, epirubicin, idarubicin, and valrubicin, mitotic inhibitors such as the vinca alkaloids vinblastine, vinvesir, vincristine, vindesine, and vinorelbine, enzymes such as L-asparaginase and PEG-L-asparaginase, microtubule polymer stabilizers such as the taxanes paclitaxel and docetaxel, topisomerase I inhibitors such as the camptothecins irinotecan and topotecan, and topoisomerase II inhibitors such as podophyllotoxin, amsacrine, etoposide, teniposide, losoxantrone and actinomycin; hormones and hormone antagonists, including androgens such as fluoxymesterone and testolactone, antiandrogens such as bicalutamide, cyproterone, flutamide, and nilutamide, corticosteroids such as dexamethasone and prednisone, aromatase inhibitors such as aminoglutethimide, anastrozole, exemestane, formestane, and letrozole: estrogens such as diethylstilbestrol, antiestrogens such as fulvestrant, raloxifene, tamoxifen, and toremifine, luteinising hormone-releasing hormone (LHRH) agonists and antagonists such as abarelix, buserelin, goserelin, leuprolide, histrelin, desorelin, nafarelin acetate and triptorelin, progestins such as medroxyprogesterone acetate and megestrol acetate, and thyroid hormones such as levothyroxine and liothyronine; PKB pathway inhibitors, including perifosine, enzastaurin hydrochloride, and triciribine, P13K inhibitors such as semaphore and SF1126, and MTOR inhibitors such as rapamycin and analogues; CDK inhibitors, including seliciclib, alvocidib, and 7-hydroxystaurosporine; COX-2 inhibitors, including celecoxib; HDAC inhibitiors, including trichostatin A, suberoylanilide hydroxamic acid, and chlamydocin; DNA methylase inhibitors, including temozolomide, and miscellaneous agents, including altretamine, arsenic trioxide, thalidomide, lenalidomide, gallium nitrate, levamisole, mitotane, hydroxyurea, octreotide, procarbazine, suramin, photodynamic compounds such as methoxsalen and sodium porfimer, and proteasome inhibitors such as bortezomib: molecular targeted therapy agents including: functional therapeutic agents, including gene therapy agents, antisense therapy agents,tyrosine kinase inhibitors such as erlotinib hydrochloride, gefitinib, imatinib mesylate, and semaxanib, Raf inhibitors such as sorafenib, and gene expression modulators such as

the retinoids and rexinoids, for example adapalene, bexarotene, trans-retinoic acid, 9-cis-retinoic acid, and N-(4-hydroxyphenyl)retinamide; and phenotype-directed therapy agents, including monoclonal antibodies such as alemtuzumab, bevacizumab, cetuximab, ibritumomab tiuxetan, rituximab, and trastuzumab, immunotoxins such as gemtuzumab ozogamicin, radioimmunoconjugates such as I-tositumobab, and cancer vaccines; Biologic therapy agents including: interferons such as interferon-[alpha]2a and interferon-[alpha]2b, and interleukins such as aldesleukin, denileukin diftitox, and oprelvekin anticancer therapies involving the use of protective or adjunctive agents, including:cytoprotective agents such as amifostine, and dexrazoxane, phosphonates such as pamidronate and zoledronic acid, and stimulating factors such as epoetin, darbeopetin, filgrastim, PEG-filgrastim, and sargramostim; and Axl inhibitor such as 1-(6,7-dihydro-5*H*-benzo[6,7]cyclohepta[1,2-*c*]pyridazin-3-yl)-N^3-((7-(*S*)-pyrrolidin-1-yl)-6,7,8,9-tetrahydro-5*H*-benzo[7]annulene-2-yl)-1*H*-1,2,4-triazole-3,5-diamine; or further combination chemotherapeutic regimens, such as combinations of carboplatin/paclitaxel, capecitabine/docetaxel, fluorauracil/levamisole, fluorauracil/leucovorin, methotrexate/leucovorin, and trastuzumab/paclitaxel, alone or in further combination with carboplatin, and the like.

37. A cell line which is sensitive to inhibitors of EMT, the cell line having a level of Akt3 expression that is just insufficient to prevent EMT, optionally wherein the cell line is a human cell line.

38. A method of identifying a compound which inhibits EMT, the method comprising contacting a cell from a cell line according to claim 37 with a test compound and determining inhibition of Akt3 activity in the cell.

39. Use of Akt3 as a biomarker for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a subject, optionally wherein an increase in the expression and/or activation of Akt3 is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

40. Use of Akt3 as a biomarker for detecting the expression and/or activation of Axl, wherein an increase in the expression and/or activation of Akt3 is indicative of an increase in the expression and/or activation of Axl.

41. A method for detecting the occurrence of epithelial-to-mesenchymal transition (EMT) in a sample, said method comprising

determining the expression level or activation of Akt3 in a sample isolated from a cell, group of cells, an animal model or human as compared to a control sample, wherein an increase in the expression level or activation of Akt3 relative to the control sample is indicative of the occurrence of epithelial-to-mesenchymal transition (EMT).

42. A method for identifying an agent capable of inhibiting or reversing epithelial-to mesenchymal transition (EMT) , said method comprising administering said agent to an *in vitro* cell or group of cells, or animal model, and monitoring the activation and/or the expression of Akt3, optionally wherein the method further comprises:

(i) administering the agent to a cell, group of cells or an animal model, not a human; and

(ii) measuring Akt3 expression and/or Akt3 activation in samples derived from the treated and the untreated cells or animal model; and

(iii) detecting an increase in the expression and/or activation of Akt3 in the treated sample as compared to the untreated sample as an indication of the ability to inhibit or reverse epithelial-to-mesenchymal transition (EMT).

43. A method according to claim 42, wherein the animal model is not a human.

44. A use or method according to any one of claims 39 to 43 wherein the level of expression of Akt3 is assessed by determining:

(i) the copy number of the gene encoding Akt3 relative to a control sample, wherein an increase in the copy number indicates an increased level of expression of Akt3; and/or

(ii) the level of Akt3 protein or mRNA.

45. A use or method according to any one of claims 39 to 44, wherein Akt3 activity is assessed by determining:

(i) phosphorylation of Akt3, wherein phosphorylation of Akt3 indicates Akt3 activity, optionally wherein Akt3 phosphorylation is determined at Serine 472; and/or

(ii) the intracellular localisation of Akt3 protein, wherein localisation in the nucleus indicates active Akt3.