(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 February 2002 (14.02.2002)

PCT

(10) International Publication Number
WO 02/012447 A3

(51) International Patent Classification[7]: C12N 5/06, 5/08, 5/10, A61K 39/00, C12Q 1/68, 1/02, A01K 67/027, A61K 39/395, 49/00

(21) International Application Number: PCT/US01/24243

(22) International Filing Date: 2 August 2001 (02.08.2001)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/222,794	3 August 2000 (03.08.2000)	US
60/240,317	13 October 2000 (13.10.2000)	US
09/920,517	1 August 2001 (01.08.2001)	US

(71) Applicant *(for all designated States except US)*: **REGENTS OF THE UNIVERSITY OF MICHIGAN** [US/US]; Wolverine Tower, Room 2071, 3003 S. State Street, Ann Arbor, MI 48109-1280 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **CLARKE, Michael, F.** [US/US]; 3377 Craig Road, Ann Arbor, MI 48103 (US). **MORRISON, Sean, J.** [US/US]; 3513 Barton Farm, Ann Arbor, MI 48105 (US). **WICHA, Max, S.** [US/US]; 2865 Parkridge Street, Ann Arbor, MI 48109 (US). **AL-HAJJ, Muhammad** [US/US]; 2035 Commere, Apt #316, Ann Arbor, MI 48103 (US).

(74) Agent: **PRINCE, John, T.**; McDermott, Will & Emery, 28 State Street, Boston, MA 02109 (US).

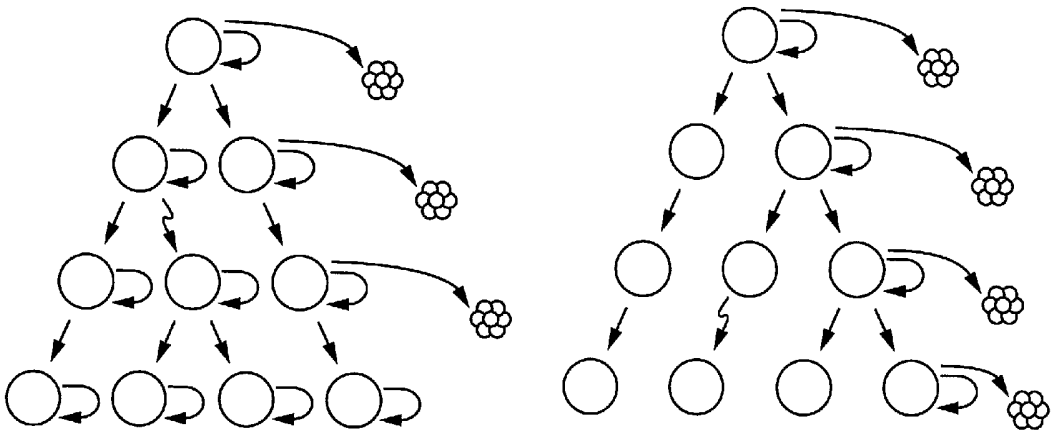
(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *with amended claims and statement*

[Continued on next page]

(54) Title: ISOLATION AND USE OF SOLID TUMOR STEM CELLS



(57) Abstract: A small percentage of cells within an established solid tumor have the properties of stem cells. These solid tumor stem cells give rise both to more tumor stem cells and to the majority of cells in the tumor that have lost the capacity for extensive proliferation and the ability to give rise to new tumors. Thus, solid tumor heterogeneity reflects the presence of tumor cell progeny arising from a solid tumor stem cell. We have developed a xenograft model in which we have been able to establish tumors from primary tumors via injection of tumors in the mammary gland of severely immunodeficient mice. These xenograft assay have allowed us to do biological and molecular assays to characterize clonogenic solid tumor stem cells. We have also developed evidence that strongly implicates the Notch pathway, especially Notch 4, as playing a central pathway in carcinogenesis.

WO 02/012447 A3

(88) Date of publication of the international search report:
24 April 2003

Date of publication of the amended claims and statement:
3 July 2003

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

AMENDED CLAIMS

[Received by the International Bureau on 21 January 2003 (21.01.03): new claims 186-449 added; remaining claims unchanged; (51 pages)]

1. An isolated solid tumor stem cell, wherein:
   (a) the solid tumor stem cell is derived from a solid tumor; and
   (b) the solid tumor stem cell is tumorigenic.

2. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell expresses at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

3. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell expresses the cell surface marker CD44.

4. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell expresses the cell surface marker epithelial specific antigen (ESA).

5. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell expresses the cell surface marker B38.1.

6. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell expresses lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

7. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell does not express detectable levels of one or more LINEAGE markers, wherein a LINEAGE marker is selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

8. The isolated solid tumor stem cell of claim 7, wherein the solid tumor stem cell does not express detectable levels LINEAGE markers, wherein the LINEAGE marker comprises CD2, CD3, CD14, CD16, and CD64.

9. The isolated solid tumor stem cell of claim 8, wherein the LINEAGE markers further comprise CD10, CD31, and CD140b.

10. The isolated solid tumor stem cell of claim 1, wherein the solid tumor is a sarcoma or an epithelial cancer.

11. The isolated solid tumor stem cell of claim 10, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

12. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell contains a polynucleotide vector.

13. The isolated solid tumor stem cell of claim 12, wherein the polynucleotide vector is a viral vector or a plasmid.

14. The isolated solid tumor stem cell of claim 12, wherein the polynucleotide vector contains a reporter polynucleotide.

15. The isolated solid tumor stem cell of claim 14, wherein the reporter polynucleotide is provides a detectable signal when active in a solid tumor stem cell.

16. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell further comprises a recombinant polynucleotide.

17. The isolated solid tumor stem cell of claim 16, wherein the recombinant polynucleotide is integrated into a chromosome of the solid tumor stem cell.

18. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell is introduced into a host mammal.

19. The isolated solid tumor stem cell of claim 18, wherein the solid tumor stem cell forms a new tumor upon transplantation into the host animal.

20. The isolated solid tumor stem cell of claim 19, wherein the animal is an immunocompromised animal.

21. The isolated solid tumor stem cell of claim 19, wherein the animal is a mammal.

22. The isolated solid tumor stem cell of claim 21, wherein the mammal is an immunocompromised mammal.

23. The isolated solid tumor stem cell of claim 21, wherein the mammal is a mouse.

24. The isolated solid tumor stem cell of claim 23, wherein the mouse is an immunocompromised mouse.

25. The isolated solid tumor stem cell of claim 24, wherein the immunocompromised mouse is selected from the group consisting of nude mouse, SCID mouse, NOD/SCID mouse, Beige/SCID mouse; and β2 microglobin deficient NOD/SCID mouse.

26. The isolated solid tumor stem cell of claim 1, further comprising a culture medium, in which culture medium the solid tumor stem cell is situated.

27. The isolated solid tumor stem cell of claim 26, wherein the culture medium comprises a Notch ligand.

28. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell is affixed to a substrate.

29. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell has been treated to reduce proliferation.

30. The isolated solid tumor stem cell of claim 1, wherein the solid tumor stem cell has been treated to increase proliferation.

31. An enriched population of solid tumor stem cells, wherein:
    (a) the tumor cells are derived from a solid tumor;
    (b) the solid tumor stem cells are tumorigenic; and
    (c) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells.

32. The enriched population of claim 31, wherein the solid tumor is a sarcoma or an epithelial cancer.

33. The enriched population of claim 32, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

34. The enriched population of claim 31, wherein the solid tumor stem cells in the enriched population express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

35. The enriched population of claim 31, wherein the solid tumor stem cells in the enriched population express the cell surface marker CD44.

36. The enriched population of claim 31, wherein the solid tumor stem cells in the enriched population express the cell surface marker epithelial specific antigen (ESA).

37. The enriched population of claim 31, wherein the solid tumor stem cells in the enriched population express the cell surface marker B38.1.

38. The enriched population of claim 31, wherein the solid tumor stem cells in the enriched population express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

39. The enriched population of claim 31, wherein solid tumor stem cells in the enriched population fail to express at least one lineage marker selected from the group consisting of CD2, CD3, CD14, CD16, CD45, CD64, and CD140b.

40. The enriched population of claim 31, wherein the enrichment is in the ability to form new tumors relative to unfractionated tumor cells.

41. The enriched population of claim 31, wherein the population is at least 5-fold enriched.

42. The enriched population of claim 31, wherein the population is at least 10-fold enriched.

43. The enriched population of claim 31, wherein the population is at least 50-fold enriched.

44. A population of cells that have been enriched for non-tumorigenic solid tumor cells, wherein:
(a) the population is derived from a solid tumor; and
(b) the population is depleted for the ability to form tumors relative to unfractionated solid tumor cells.

45. The enriched population of claim 44, wherein the solid tumor is a sarcoma or an epithelial cancer.

46. The enriched population of claim 45, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

47. A method for enriching a population of cells for solid tumor stem cells, comprising the steps of:
(a) dissociating a solid tumor to form a cell suspension;
(b) contacting the dissociated cells with at least one reagent, wherein the reagent either selectively binds to a solid tumor stem cell positive marker or negative marker; and
(c) selecting cells that bind to the reagent that selectively binds to a positive marker and/or that do not bind to the reagent that selectively binds to a negative marker, wherein the selected cells are enriched in tumor stem cells as compared with the unfractionated population of solid tumor cells.

48. The method of claim 47, wherein the solid tumor is a sarcoma or epithelial cancer.

49. The method of claim 48, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

50. The method of claim 47, wherein the reagent is an antibody or a lectin.

51. The method of claim 47, wherein the reagent is conjugated to a fluorochrome or to magnetic particles.

52. The method of claim 47, wherein the solid tumor stem cell positive marker is a marker selected from the group consisting of CD44, B38.1 and ESA.

53. The method of claim 47, wherein the solid tumor stem cell negative marker is a marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

54. The method of claim 47, wherein the cell selection is performed is by flow cytometry, fluorescence activated cell sorting, panning, affinity column separation, and/or magnetic selection.

55. The method of claim 47, wherein steps (b) and (c) comprise:

(b) contacting the dissociated cells with a combination of reagents, wherein each reagent in the combination either selectively binds to either a solid tumor stem cell positive marker or negative marker; and

(c) selecting cells that bind to reagents that selectively bind to the positive marker or that do not bind to reagents that selectively bind to the negative marker or a combination thereof, wherein the selected cells are enriched in tumor stem cells as compared with the population of unfractionated cells.

56. The method of claim 47, further comprising the step of:

(d) isolating the selected solid tumor stem cells.

57. The method of claim 47, further comprising the steps of:

(d) introducing at least one selected cell to a culture medium that supports the growth of tumor stem cells; and

(e) proliferating the selected cell in the culture medium.

58. The method of claim 57, further comprising the step of:

(f) introducing the proliferated cell into a host mammal.

59. The method of claim 57, further comprising the steps of:

(f) contacting the proliferated cell with a test compound; and

(g) determining the effect of the test compound on the proliferated cell.

60. The method of claim 57, further comprising the steps of:

(f) mixing a population of non-tumorigenic tumor cells with the solid tumor stem cells in culture, wherein the population of non-tumorigenic tumor cells

(i) is derived from a solid tumor;

(ii) is depleted for the ability to form tumors relative to unfractionated solid tumor cells.

61. The method of claim 60, further comprising the steps of:

(g) transplanting the mixture into a host animal.

62. The method of claim 60, further comprising the steps of:

(g) analyzing the mixture for an increase or decrease in the ability of the solid tumor stem cells to survive or proliferate.

63. The method of claim 57, further comprising the step of:

(f) isolating the proliferated solid tumor stem cell.

64. A method for stimulating an immune response to a solid tumor stem cell, comprising the steps of:

(a) obtaining an enriched population of solid tumor stem cells; wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

(b) treating the population to prevent cell replication; and

(c) administering the treated cell to a human or animal subject in an amount effective for inducing an immune response to solid tumor stem cells.

65. The method of claim 64, wherein the solid tumor is a sarcoma or epithelial cancer.

66. The method of claim 65, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

67. The method of claim 64, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

68. The method of claim 64, wherein the treatment kills the solid tumor stem cells.

69. The method of claim 64, wherein the administration is by injection or by oral administration.

70. The method of claim 64, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

71. The method of claim 64, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

72. The method of claim 64, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

73. The method of claim 64, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker B38.1.

74. The method of claim 64, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

75. The method of claim 64, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

76. The method of claim 64, further comprising the step of:
(d) obtaining antibodies or antibody secreting hybridomas from the human or animal subject.

77. The method of claim 76, further comprising the step of:
(e) testing the obtained antibody for the ability to specifically bind to solid tumor stem cells.

78. The method of claim 76, further comprising the step of:
(e) testing the obtained antibody for the ability to bind to a polypeptide present on solid tumor stem cells.

79. The method of claim 76, further comprising the step of:
(e) immunologically identifying a polypeptide present on solid tumor stem cells.

80. The method of claim 76, further comprising the step of:
(e) immunologically identifying a polynucleotide encoding a polypeptide present on solid tumor stem cells.

81. The method of claim 76, further comprising the step of:

(e) testing the obtained antibody for the ability to reduce tumor growth.

82. A method for stimulating an immune response to a solid tumor stem cell, comprising the steps of:

(a) obtaining an enriched population of solid tumor stem cells; wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

(b) mixing the tumor stem cells in an *in vitro* culture with immune effector cells;

(c) removing the immune effector cells from the culture; and

(d) transplanting the immune effector cells into a host animal in a dose that is effective to stimulate an immune response in the animal.

83. The method of claim 82, wherein the solid tumor is a sarcoma or epithelial cancer.

84. The method of claim 83, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

85. The method of claim 82, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

86. The method of claim 82, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

87. The method of claim 82, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker B38.1.

88. The method of claim 82, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

89. The method of claim 82, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

90. The method of claim 82, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

91. A purified population of polynucleotides, wherein the polynucleotides have been purified from an enriched population of solid tumor stem cells, wherein:
(a) the tumor cells are derived from a solid tumor;
(b) the solid tumor stem cells are tumorigenic; and
(c) the solid tumor stem cell population is enriched at least at least 2-fold relative to unfractionated tumor cells.

92. The polynucleotide population of claim 91, wherein the solid tumor is a sarcoma or an epithelial cancer.

93. The polynucleotide population of claim 92, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

94. The polynucleotide population of claim 91, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

95. The polynucleotide population of claim 91, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

96. The polynucleotide population of claim 91, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

97. The polynucleotide population of claim 91, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cell expresses the cell surface marker B38.1.

98. The polynucleotide population of claim 91, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

99. The polynucleotide population of claim 91, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

100. The polynucleotide population of claim 91, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

101. The polynucleotide population of claim 91, wherein the polynucleotides are affixed to a solid surface.

102. The polynucleotide population of claim 101, wherein the polynucleotides are affixed to the solid surface in an orderly array.

103. The polynucleotide population of claim 102, wherein the orderly array is a microarray.

104. The polynucleotide population of claim 91, wherein the polynucleotides are in a cDNA library.

105. The polynucleotide population of claim 91, wherein the polynucleotides have been amplified.

106. The polynucleotide population of claim 91, wherein the polynucleotides are labeled.

107. The polynucleotide population of claim 91, wherein the polynucleotides are used as a hybridization probe.

108. The polynucleotide population of claim 107, further comprising a microarray of polynucleotide sequences.

109. A purified population of polypeptides, wherein the polypeptides have been purified from an enriched population of solid tumor stem cells, wherein:
(a) the tumor cells are derived from a solid tumor;
(b) the solid tumor stem cells are tumorigenic; and
(c) the solid tumor stem cell population is enriched at least at least 2-fold relative to unfractionated tumor cells.

110. The polypeptide population of claim 109, wherein the solid tumor is a sarcoma or an epithelial cancer.

111. The polypeptide population of claim 110, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

112. The polypeptide population of claim 109, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

113. The polypeptide population of claim 109, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

114. The polypeptide population of claim 109, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker CD44.

115. The polypeptide population of claim 109, wherein, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

116. The polypeptide population of claim 109, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

117. The polypeptide population of claim 109, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

118. The polypeptide population of claim 109, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

119. The polypeptide population of claim 109, wherein the polypeptides are affixed to a solid surface.

120. The polypeptide population of claim 109, wherein the polypeptides are affixed to the solid surface in an orderly array.

121. The polypeptide population of claim 120, wherein the orderly array is a microarray.

122. The polypeptide population of claim 109, wherein the polypeptides are labeled.

123. The polypeptide population of claim 109, wherein the polypeptides are used as a probe.

124. The polypeptide population of claim 123, further comprising a microarray of components, wherein the components are selected from the group consisting of cells, polynucleotides, polypeptides, and test compounds.

125. A method for analyzing a population of cells enriched for solid tumor stem cells for gene expression patterns, comprising the steps of:

(a) obtaining an population of cells enriched for solid tumor stem cells; wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and

(b) analyzing the population of cells for gene expression patterns.

126. The method of claim 125, wherein the analysis is by a method selected from the group consisting of resequencing, high throughput screening, use of a microarray, use of analytical software for data collection and storage, use of analytical software for flexible formatting of data output, use of analytical software for statistical analysis of individual spot intensities to provide grouping and cluster analyses, and use of analytical software for linkage to external databases.

127. The method of claim 125 wherein the solid tumor is a sarcoma or an epithelial cancer.

128. The method of claim 127, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

129. The method of claim 125, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

130. The method of claim 125, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

131. The method of claim 125, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

132. The method of claim 125, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

133. The method of claim 125, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

134. The method of claim 125, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

135. The method of claim 125, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

136. A method for analyzing a population of cells enriched for solid tumor stem cells for protein expression patterns, comprising the steps of:

(a) obtaining an population of cells enriched for solid tumor stem cells; wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and

(b) analyzing the population of cells for protein expression patterns.

137. The method of claim 136, wherein the analysis is by a method selected from the group consisting of mass spectrometry, high throughput screening, use of a microarray, use of analytical software for data collection and storage, use of analytical software for flexible formatting of data output, use of analytical software for statistical analysis of individual spot intensities to provide grouping and cluster analyses, and use of analytical software for linkage to external databases.

138. The method of claim 136, wherein the solid tumor is a sarcoma or an epithelial cancer.

139. The method of claim 138, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

140. The method of claim 136, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

141. The method of claim 136, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

142. The method of claim 136, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

143. The method of claim 136, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

144. The method of claim 136, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker B38.1.

145. The method of claim 136, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

146. The method of claim 136, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

147. A method for determining the effect of a test compound on a solid tumor stem cell, comprising the steps of:

(a) obtaining an enriched population of solid tumor stem cells, wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

(b) contacting the obtained cells with the test compound; and

(c) determining the response of the contacted cells to the test compound.

148. The method of claim 147, wherein the solid tumor is a sarcoma or an epithelial cancer.

149. The method of claim 148, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

150. The method of claim 147, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

151. The method of claim 147, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

152. The method of claim 147, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

153. The method of claim 147, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

154. The method of claim 147, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

155. The method of claim 147, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

156. The method of claim 147, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

157. The method of claim 147, wherein the solid tumor stem cells are localized in a manner selected from the group consisting of in monolayers in culture, in suspension in culture, and affixed to a solid surface.

158. The method of claim 147, wherein the contacting is effected at more than one concentration of the test compound being tested.

159. The method of claim 147, wherein the contacting is effected using microfluidic methods.

160. The method of claim 147, wherein the determination of the response of the contacted cells to the test compound comprises assaying for an effect selected from the group consisting of tumor formation, tumor growth, tumor stem cell proliferation, tumor cell survival, tumor cell cycle status, and tumor stem cell survival.

161. The method of claim 147, wherein the test compound is attached to a solid surface.

162. The method of claim 161, wherein the test compound is attached to a solid surface as a microarray.

163. The method of claim 147, wherein the test compound is in a set of other molecules.

164. The method of claim 147, wherein the test compound is in an array of other molecules.

165. The method of claim 147, further comprising the step of.

(d) identifying the target in the contacted cells with which the test compound interacts.

166. A method for determining the effect of a test compound on a solid tumor stem cell, comprising the steps of:

(a) contacting a population of solid tumor cells with the test compound;

(b) obtaining an enriched population of solid tumor stem cells from the contacted solid tumor cells, wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and

(c) determining the response of the contacted cells to the test compound.

167. The method of claim 166, wherein the solid tumor is a sarcoma or an epithelial cancer.

168. The method of claim 167, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

169. The method of claim 166, wherein the solid tumor is a sarcoma or an epithelial cancer.

170. The method of claim 166, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

171. The method of claim 166, wherein the solid tumor cells are *in vivo*.

172. The method of claim 166, wherein the solid tumor cells are *in vitro*.

173. The method of claim 166, wherein the solid tumor cells are in raw tumor tissue.

174. The method of claim 166, wherein the solid tumor cells are a cell line.

175. The method of claim 166, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

176. The method of claim 166, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

177. The method of claim 166, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

178. The method of claim 166, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

179. The method of claim 166, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

180. The method of claim 166, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

181. The method of claim 166, wherein the solid tumor stem cells are localized in a manner selected from the group consisting of in monolayers in culture, in suspension in culture, and affixed to a solid surface.

182. The method of claim 166, wherein the contacting is effected at more than one concentration of the test compound being tested.

183. The method of claim 166, wherein the contacting is effected using microfluidic methods.

184. The method of claim 166, wherein the determination of the response of the contacted cells to the test compound comprises assaying for an effect selected from the group consisting of tumor formation, tumor growth, tumor stem cell proliferation, tumor cell survival, tumor cell cycle status, and tumor stem cell survival.

185. The method of claim 166, wherein the test compound is attached to a solid surface.

186. The method of claim 185, wherein the test compound is attached to a solid surface as a microarray.

187. The method of claim 166, wherein the test compound is in a set of other molecules.

188. The method of claim 166, wherein the test compound is in an array of other molecules.

189. The method of claim 166, further comprising the step of.

(d) identifying the target in the contacted cells with which the test compound interacts.

190. A method for determining the effect of a test compound on a solid tumor stem cell, comprising the steps of:

(a) obtaining an enriched population of solid tumor stem cells, wherein;

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

(b) transplanting the obtained cells into an animal;

(c) administering a test compound to the animal; and

(d) determining the response of the transplanted solid tumor stem cells to the test compound.

191. The method of claim 190, wherein the animal is an immunocompromised animal.

192. The method of claim 190, wherein the animal is a mammal.

193. The method of claim 192, wherein the mammal is an immunocompromised mammal.

194. The method of claim 192, wherein the mammal is a mouse.

195. The method of claim 194, wherein the mouse is an immunocompromised mouse.

196. The method of claim 195, wherein the immunocompromised mouse is selected from the group consisting of nude mouse, SCID mouse, NOD/SCID mouse, Beige/SCID mouse; and β2 microglobin deficient NOD/SCID mouse.

197. The method of claim 190, wherein the solid tumor is a sarcoma or an epithelial cancer.

198. The method of claim 197, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

199. The method of claim 190, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

200. The method of claim 190, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

201. The method of claim 190, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

202. The method of claim 190, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

203. The method of claim 190, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

204. The method of claim 190, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

205. The method of claim 190, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

206. A method for determining the effect of a test compound on a solid tumor stem cell, comprising the steps of:

(a) administering a test compound to an animal;

(b) obtaining from the animal an enriched population of solid tumor stem cells, wherein;

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and

(c) determining the response of the solid tumor stem cells to the test compound.

207. The method of claim 206, wherein the animal is an immunocompromised animal.

208. The method of claim 206, wherein the animal is a mammal.

209. The method of claim 208, wherein the mammal is an immunocompromised mammal.

210. The method of claim 208, wherein the mammal is a mouse.

211. The method of claim 210, wherein the mouse is an immunocompromised mouse.

212. The method of claim 211, wherein the immunocompromised mouse is selected from the group consisting of nude mouse, SCID mouse, NOD/SCID mouse, Beige/SCID mouse; and β2 microglobin deficient NOD/SCID mouse.

213. The method of claim 206, wherein the solid tumor is a sarcoma or an epithelial cancer.

214. The method of claim 213, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

215. The method of claim 206, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

216. The method of claim 206, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

217. The method of claim 206, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express the cell surface marker CD44.

218. The method of claim 206, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

219. The method of claim 206, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express the cell surface marker B38.1.

220. The method of claim 206, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

221. The method of claim 206, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

222. A method for screening for a test compound that specifically binds to a solid tumor stem cell, comprising the steps of:

(a) obtaining an enriched population of solid tumor stem cells, wherein;

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

(b) contacting the enriched population of solid tumor stem cells with a test compound under conditions suitable to allow complex formation; and

(c) detecting complex formation between the test compound and a solid tumor stem cell, wherein the presence of the complex identifies the test compound as specifically binding the tumor stem cell.

223. The method of claim 222, wherein the solid tumor is a sarcoma or an epithelial cancer.

224. The method of claim 223, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

225. The method of claim 222, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

226. The method of claim 222, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

227. The method of claim 222, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

228. The method of claim 222, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

229. The method of claim 222, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

230. The method of claim 222, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

231. The method of claim 222, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

232. A method for screening for a test compound that specifically binds to a solid tumor stem cell, comprising the steps of:

(a) contacting a population of solid tumor cells with a test compound under conditions suitable to allow complex formation; and

(b) obtaining an enriched population of solid tumor stem cells from the contacted solid tumor cells, wherein;

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

(c) detecting complex formation between the test compound and a solid tumor stem cell, wherein the presence of the complex identifies the test compound as specifically binding the tumor stem cell.

233. The method of claim 232, wherein the solid tumor is a sarcoma or an epithelial cancer.

234. The method of claim 233, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

235. The method of claim 232, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

236. The method of claim 232, wherein the solid tumor cells are *in vivo*.

237. The method of claim 232, wherein the solid tumor cells are *in vitro*.

238. The method of claim 232, wherein the solid tumor cells are in raw tumor tissue.

239. The method of claim 232, wherein the solid tumor cells are a cell line.

240. The method of claim 232, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

241. The method of claim 232, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express the cell surface marker CD44.

242. The method of claim 232, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

243. The method of claim 232, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express the cell surface marker B38.1.

244. The method of claim 232, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

245. The method of claim 232, wherein, in the enriched population of solid tumor stem cells of (b), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

246. A method for diagnosing the presence of solid tumor stem cells, comprising the steps of:
(a) contacting the cells from a solid tumor with a reagent that binds to a positive marker for solid tumor stem cells; and
(b) detecting the contact between the reagent and the cells from the solid tumor, wherein an increased detection of the number of contacted cells as compared with the number of contacted cells in a benign tumor identifies the tumor as containing solid tumor stem cells.

247. The method of claim 246, wherein the detection is by flow-cytometry or immunohistochemistry.

248. The method of claim 247, wherein the solid tumor is a sarcoma or an epithelial cancer.

249. The method of claim 246, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

250. The method of claim 246, wherein the positive marker is a marker selected from the group consisting of CD44, B38.1 and ESA.

251. An *in vitro* method for the proliferation of a tumor stem cells, comprising the steps of:
(a) obtaining an enriched population of solid tumor stem cells, wherein;
(i) the tumor cells are derived from a solid tumor;
(ii) the solid tumor stem cells are tumorigenic; and
(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and
(b) proliferating the obtained cells in a culture medium.

252. The method of claim 251, wherein the solid tumor is a sarcoma or an epithelial cancer.

253. The method of claim 252, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

254. The method of claim 251, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

255. The method of claim 251, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

256. The method of claim 251, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

257. The method of claim 251, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

258. The method of claim 251, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker B38.1.

259. The method of claim 251, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

260. The method of claim 251, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

261. An *in vivo* method for the proliferation of a tumor stem cells, comprising the steps of:

(a) obtaining an enriched population of solid tumor stem cells, wherein;

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and

(b) transplanting the isolated cell into a host mammal under conditions that allow the proliferation of solid tumor stem cells in the host mammal.

262. The method of claim 261, wherein the solid tumor is a sarcoma or an epithelial cancer.

263. The method of claim 262, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

264. The method of claim 261, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

265. The method of claim 261, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

266. The method of claim 261, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

267. The method of claim 261, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

268. The method of claim 261, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

269. The method of claim 261, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

270. The method of claim 261, further comprising the step of:
(c) isolating the proliferated tumor cells from the host mammal.

271. A method for producing genetically modified tumor stem cells, comprising the steps of:
(a) obtaining an enriched population of solid tumor stem cells, wherein:
(i) the tumor cells are derived from a solid tumor;
(ii) the solid tumor stem cells are tumorigenic;
(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;
(b) genetically modifying the obtained cells.

272. The method of claim 271, wherein the solid tumor is a sarcoma or an epithelial cancer.

273. The method of claim 262, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

274. The method of claim 271, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

275. The method of claim 271, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

276. The method of claim 271, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

277. The method of claim 271, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

278. The method of claim 271, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

279. The method of claim 271, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

280. The method of claim 271, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

281. The method of claim 271, in which the genetic modification is performed *in vitro*.

282. The method of claim 271, in which the genetic modification is performed *in vivo*.

283. The method of claim 271, wherein the genetic modification is the introduction of a plasmid into the solid tumor stem cell.

284. The method of claim 271, wherein the genetic modification is the introduction of a viral vector into the solid tumor stem cell.

285. The method of claim 271, in which the virus has been modified to express a protein that recognizes an antigen on the solid tumor stem cell, thus specifically targeting the virus to solid tumor stem cell.

286. The method of claim 271, further comprising the step of:

(c) examining the effect of the genetic modification on tumor formation, tumor growth, tumor cell proliferation, tumor cell survival, tumor stem cell survival, tumor stem cell proliferation, tumor cell cycle status, and tumor stem cell frequency.

287. A method for producing genetically modified tumor stem cells, comprising the steps of:

(a) genetically modifying cells of a population of solid tumor cells; and

(b) obtaining an enriched population of solid tumor stem cells from the genetically modified solid tumor cells, wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic;

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

288. The method of claim 287, wherein the solid tumor is a sarcoma or an epithelial cancer.

289. The method of claim 288, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

290. The method of claim 287, wherein the obtained enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

291. The method of claim 287, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

292. The method of claim 287, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

293. The method of claim 287, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

294. The method of claim 287, wherein, in the enriched population of solid tumor stem cells, the solid tumor stem cells express the cell surface marker B38.1.

295. The method of claim 287, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

296. The method of claim 287, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

297. The method of claim 287, in which the genetic modification is performed *in vitro*.

298. The method of claim 287, in which the genetic modification is performed *in vivo*.

299. The method of claim 287, wherein the genetic modification is the introduction of a plasmid into the solid tumor cell.

300. The method of claim 287, wherein the genetic modification is the introduction of a viral vector into the solid tumor cell.

301. The method of claim 287, in which the virus has been modified to express a protein that recognizes an antigen on the solid tumor stem cell, thus specifically targeting the virus to solid tumor cell.

302. The method of claim 287, further comprising the step of:

(c) examining the effect of the genetic modification on tumor formation, tumor growth, tumor cell proliferation, tumor cell survival, tumor stem cell survival, tumor stem cell proliferation, tumor cell cycle status, and tumor stem cell frequency.

303. An *in vivo* method for proliferating a population of cancer cells, comprising:
(a) introducing an enriched population of solid tumor stem cells into a host animal; wherein:
(i) the tumor cells are derived from a solid tumor;
(ii) the solid tumor stem cells are tumorigenic;
(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;
(b) proliferating the solid tumor stem cells in the host animal; and
(c) purifying the proliferated solid tumor stem cells from the host animal.

304. The method of claim 303, wherein the animal is an immunocompromised animal.

305. The method of claim 303, wherein the animal is a mammal.

306. The method of claim 305, wherein the mammal is an immunocompromised mammal.

307. The method of claim 305, wherein the mammal is a mouse.

308. The method of claim 307, wherein the mouse is an immunocompromised mouse.

309. The method of claim 308, wherein the immunocompromised mouse is selected from the group consisting of nude mouse, SCID mouse, NOD/SCID mouse, Beige/SCID mouse; and β2 microglobin deficient NOD/SCID mouse.

310. The method of claim 303, wherein the solid tumor is a sarcoma or an epithelial cancer.

311. The method of claim 310, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

312. The method of claim 303, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

313. The method of claim 303, wherein, in the enriched population of solid tumor stem cells (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

314. The method of claim 303, wherein, in the enriched population of solid tumor stem cells (a), the solid tumor stem cells express the cell surface marker CD44.

315. The method of claim 303, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

316. The method of claim 303, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker B38.1.

317. The method of claim 303, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

318. The method of claim 303, wherein the solid tumor stem cell fails to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

319. The method of claim 303, wherein the cells are introduced subcutaneously or into the mammary fat pad.

320. The method of claim 303, wherein tumors grow in the mammal in greater than 75% of the introductions.

321. The method of claim 303, wherein tumors grow in the mammal in greater than 90% of the introductions.

322. The method of claim 303, wherein the population of cells has been enriched at least 50-fold.

323. The method of claim 303, wherein the population of cells has been enriched at least 5-fold.

324. The method of claim 303, wherein the population of cells has been enriched at least 10-fold.

325. The method of claim 303, wherein the mice have been further immunosuppressed by a method selected from the group consisting of administration of VP16, radiation therapy and chemotherapy.

326. The method of claim 303, further comprising:
(d) isolating an enriched population of solid tumor stem cells from the proliferated cells

327. The method of claim 326, wherein the isolation comprises the use of flow-cytometry.

328. A method for growing a solid tumor stem cell from a solid tumor, comprising the steps of:
(a) separating the cells of the solid tumor;
(b) suspending the separated tumor cells in suspension; and
(c) introducing the suspended cell into a host mammal, such that the cells in the introduced suspension forms a tumor in the host mammal.

329. The method of claim 328, wherein the solid tumor is a sarcoma or an epithelial cancer.

330. The method of claim 329, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

331. The method of claim 328, wherein the solid tumor stem cells in the suspension express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

332. The method of claim 328, wherein the solid tumor stem cells in the suspension express the cell surface marker CD44.

333. The method of claim 328, wherein the solid tumor stem cells in the suspension express the cell surface marker epithelial specific antigen (ESA).

334. The method of claim 328, wherein the solid tumor stem cells in the suspension express the cell surface marker B38.1.

335. The method of claim 328, wherein the solid tumor stem cells in the suspension lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

336. The method of claim 328, wherein the solid tumor stem cells in the suspension fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

337. A tumor bank, comprising cells derived from a single tumor, wherein the tumor has been produced by the steps of:

(a) introducing an enriched population of solid tumor stem cells into a host mammal; wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells;

(b) proliferating the introduced cells in the host mammal; and

(c) isolating the proliferated cells from the host mammal.

338. The tumor bank of claim 337, wherein the solid tumor is a sarcoma or an epithelial cancer.

339. The tumor bank of claim 338, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

340. The tumor bank of claim 337, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

341. The tumor bank of claim 337, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

342. The tumor bank of claim 337, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker CD44.

343. The tumor bank of claim 337, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

344. The tumor bank of claim 337, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker B38.1.

345. The tumor bank of claim 337, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

346. The tumor bank of claim 337, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

347. A chimeric animal, comprising:

(a) the animal; and

(b) an enriched population of solid tumor stem cells, wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic;

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and

(iv) the cells of the enriched population have been introduced into the animal.

348. The chimeric animal of claim 337, wherein the solid tumor of (b) is a sarcoma or an epithelial cancer.

349. The chimeric animal of claim 338, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

350. The chimeric animal of claim 337, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

351. The chimeric animal of claim 337, wherein, in the enriched population of solid tumor stem cells (b), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

352. The chimeric animal of claim 337, wherein, in the enriched population of solid tumor stem cells (b), the solid tumor stem cells express the cell surface marker CD44.

353. The chimeric animal of claim 337, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

354. The chimeric animal of claim 337, wherein, in the enriched population of solid tumor stem cells (b), the solid tumor stem cells express the cell surface marker B38.1.

355. The chimeric animal of claim 337, wherein, in the enriched population of solid tumor stem cells (b), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

356. The chimeric animal of claim 337, wherein, in the enriched population of solid tumor stem cells (b), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

357. The chimeric animal of claim 337, wherein the introduction is by injection.

358. The chimeric animal of claim 337, wherein the animal is an immunocompromised animal.

359. The chimeric animal of claim 337, wherein the animal is a mammal.

360. The chimeric animal of claim 349, wherein the mammal is an immunocompromised mammal.

361. The chimeric animal of claim 349, wherein the mammal is a mouse.

362. The chimeric animal of claim 351, wherein the mouse is an immunocompromised mouse.

363. The chimeric animal of claim 352, wherein the immunocompromised mouse is selected from the group consisting of nude mouse, SCID mouse, NOD/SCID mouse, Beige/SCID mouse; and β2 microglobin deficient NOD/SCID mouse.

364. An *in vivo* method for modeling a tumor treatment regime, comprising the steps of:

(a) introducing an enriched population of solid tumor stem cells into an immunocompromised mouse under conditions that allow the solid tumor stem cells to proliferate to form a tumor; wherein:

(i) the tumor cells are derived from a solid tumor;

(ii) the solid tumor stem cells are tumorigenic; and

(iii) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells; and

(b) testing the effects of treatment regimens on the solid tumor cells in the immunocompromised mouse by monitoring the effect of the treatment regimen.

365. The method of claim 354, wherein the solid tumor is a sarcoma or an epithelial cancer.

366. The method of claim 355, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

367. The method of claim 354, wherein the enriched population of solid tumor stem cells is an isolated solid tumor stem cell.

368. The method of claim 354, wherein, in the enriched population of solid tumor stem cells (a), the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

369. The method of claim 354, wherein, in the enriched population of solid tumor stem cells (a), the solid tumor stem cells express the cell surface marker CD44.

370. The method of claim 354, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

371. The method of claim 354, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express the cell surface marker B38.1.

372. The method of claim 354, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

373. The method of claim 354, wherein, in the enriched population of solid tumor stem cells of (a), the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

374. A method for reducing the size of a solid tumor, comprising the step of:
contacting the cells of the solid tumor with a therapeutically effective amount of an agent directed against a Notch 4 polypeptide.

375. The method of claim 364, wherein the therapeutically effective amount is an amount sufficient to cause cell death of or inhibit the proliferation of solid tumor stem cells in the solid tumor.

376. The method of claim 364, wherein the agent is an antibody, peptide or small molecule directed against a Notch 4 polypeptide.

377. The method of claim 366, wherein the antibody, peptide or small molecule is directed against the extracellular domain of Notch 4.

378. A method for reducing the size of a solid tumor, comprising:
contacting the cells of the solid tumor with a therapeutically effective amount of an agent that modulates the activity of a Notch 4 ligand.

379. The method of claim 37, wherein the Notch 4 ligand is selected from the group consisting of Delta 1, Delta 2, Delta-like ligand 4 (Dll4), Jagged 1 and Jagged 2.

380. The method of claim 37, wherein the agent is a Notch ligand agonist.

381. The method of claim 37, wherein the agent is a Notch ligand antagonist.

382. A method for reducing the size of a solid tumor, comprising:
contacting the cells of the solid tumor with a therapeutically effective amount of an agent that modulates the activity of Maniac Fringe.

383. The method of claim 38, wherein the agent is a Maniac Fringe agonist.

384. The method of claim 38, wherein the agent is a Maniac Fringe antagonist.

385. A method for killing or inhibiting the proliferation of solid tumor stem cells, comprising the step of:
contacting the cells of a solid tumor with an agent or combination of agents selectively targeted to the solid tumor stem cells of the solid tumor, wherein the agent or combination of agents kills or inhibits the proliferation of solid tumor stem cells.

386. The method of claim 385, further comprising the step of:
identifying the death of or the prevention of the growth of solid tumor stem cells in the solid tumor following contact by the agent or combination of agents.

387. The method of claim 385, wherein the killing is by the activation of cell death in the solid tumor stem cells.

388. The method of claim 387, wherein the cell death is apoptosis.

389. The method of claim 385, wherein the agent or combination of agents inhibits Notch-4 signaling.

390. The method of claim 385, wherein the agent is an antibody, peptide or small molecule directed against a Notch 4 polypeptide.

391. The method of claim 385, wherein the antibody, peptide or small molecule is directed against the extracellular domain of Notch 4.

392. The method of claim 385, wherein the agent or combination of agents modulates the activity of a Notch 4 ligand.

393. The method of claim 392, wherein the Notch 4 ligand is selected from the group consisting of Delta 1, Delta 2, Delta-like ligand 4 (Dll4), Jagged 1 and Jagged 2.

394. The method of claim 385, wherein the agent or combination of agents modulates the activity of Maniac Fringe.

395. The method of claim 385, wherein the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

396. The method of claim 385, wherein the solid tumor stem cells express the cell surface marker CD44.

397. The method of claim 385, wherein the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

398. The method of claim 385, wherein the solid tumor stem cells express the cell surface marker B38.1.

399. The method of claim 385, wherein the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells of the solid tumor.

400. The method of claim 385, wherein the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

401. The method of claim 385, wherein the solid tumor is an epithelial cancer or a sarcoma.

402. The method of claim 401, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

403. A method for reducing the size of a solid tumor, comprising the step of:
contacting the cells of the solid tumor *in vivo* with an agent or combination of agents selectively targeted to the solid tumor stem cells of the solid tumor, wherein the agent or combination of agents kills or inhibits the proliferation of solid tumor stem cells.

404. The method of claim 400, further comprising the step of:
identifying the death of or the prevention of the growth of solid tumor stem cells in the solid tumor following contact by the agent or combination of agents.

405. The method of claim 403, wherein the killing is by the activation of cell death in the solid tumor stem cells.

406. The method of claim 405, wherein the cell death is apoptosis.

407. The method of claim 403, wherein the agent or combination of agents inhibits Notch-4 signaling.

408. The method of claim 403, wherein the agent is an antibody, peptide or small molecule directed against a Notch 4 polypeptide.

409. The method of claim 408, wherein the antibody, peptide or small molecule is directed against the extracellular domain of Notch 4.

410. The method of claim 403, wherein the agent or combination of agents modulates the activity of a Notch ligand.

411. The method of claim 403, wherein the Notch 4 ligand is selected from the group consisting of Delta 1, Delta 2, Delta-like ligand 4 (Dll4), Jagged 1 and Jagged 2.

412. The method of claim 403, wherein the agent or combination of agents modulates the activity of Maniac Fringe.

413. The method of claim 403, wherein the solid tumor stem cells express at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

414. The method of claim 403, wherein the solid tumor stem cells express the cell surface marker CD44.

415. The method of claim 403, wherein the solid tumor stem cells express the cell surface marker epithelial specific antigen (ESA).

416. The method of claim 403, wherein the solid tumor stem cells express the cell surface marker B38.1.

417. The method of claim 403, wherein the solid tumor stem cells fail to express at least one LINEAGE marker selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

418. The method of claim 403, wherein the solid tumor stem cells express lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells of the solid tumor.

419. The method of claim 403, wherein the solid tumor is an epithelial cancer or a sarcoma.

420. The method of claim 419, wherein the epithelial cancer is a breast cancer or an ovarian cancer.

421. A method for selectively targeting a solid tumor stem cell, comprising the steps of:
(a) identifying a marker present on a solid tumor stem cell;
(b) obtaining a biomolecule or set of biomolecules that selectively binds to the marker present on the solid tumor stem cell.

422. The method of claim 421, wherein the biomolecule genetically modifies the targeted solid tumor stem cell.

423. The method of claim 422, wherein the genetic modification results in solid tumor stem cell death.

424. The method of claim 421, wherein the biomolecule or set of biomolecules comprises a bi-specific conjugate.

425. The method of claim 421, wherein the biomolecule or set of biomolecules comprises an adenoviral vector.

426. The method of claim 425, wherein the adenoviral vector is selectively targeted to a solid tumor stem cell.

427. A biomolecule or set of biomolecules that is selectively targeted to solid tumor stem cell.

428. The method of claim 427, wherein the biomolecule genetically modifies the targeted solid tumor stem cell.

429. The method of claim 428, wherein the genetic modification results in solid tumor stem cell death.

430. The method of claim 427, wherein the biomolecule or set of biomolecules comprises a bi-specific conjugate.

431. The method of claim 427, wherein the biomolecule or set of biomolecules comprises an adenoviral vector.

432. The method of claim 431, wherein the adenoviral vector is selectively targeted to a solid tumor stem cell.

433. A method for forming a tumor in an animal, comprising:

introducing a cell dose of purified solid tumor stem cells into the animal, wherein:

(a) the solid tumor stem cell is derived from a solid tumor;

(b) the solid tumor stem cell population is enriched at least 2-fold relative to unfractionated tumor cells.

434. The method of claim 433, wherein the animal is an immunocompromised animal.

435. The method of claim 433, wherein the animal is a mammal.

436. The method of claim 435, wherein the mammal is an immunocompromised mammal.

437. The method of claim 435, wherein the mammal is a mouse.

438. The method of claim 437, wherein the mouse is an immunocompromised mouse.

439. The method of claim 438, wherein the immunocompromised mouse is selected from the group consisting of nude mouse, SCID mouse, NOD/SCID mouse, Beige/SCID mouse; and β2 microglobin deficient NOD/SCID mouse.

440. The method of claim 433, wherein the number of cells in the cell dose is between about 100 cells and about $5x10^5$ cells.

441. The method of claim 433, wherein the number of cells in the cell dose is about between about 100 cells and 500 cells.

442. The method of claim 433, wherein the number of cells in the cell dose is between about 100 cells and 200 cells.

443. The method of claim 433, wherein the number of cells in the cell dose is about 100 cells.

444. The method of claim 433, wherein the solid tumor stem cell expresses at least one marker selected from the group consisting of CD44, epithelial specific antigen (ESA), and B38.1.

445. The method of claim 433, wherein the solid tumor stem cell expresses the cell surface marker CD44.

446. The method of claim 433, wherein the solid tumor stem cell expresses the cell surface marker epithelial specific antigen (ESA).

447. The method of claim 433, wherein the solid tumor stem cell expresses the cell surface marker B38.1.

448. The method of claim 433, wherein the solid tumor stem cell expresses lower levels of the marker CD24 than the mean expression of CD24 by non-tumorigenic cancer cells derived from the solid tumor.

449. The method of claim 433, wherein the solid tumor stem cell does not express detectable levels of one or more LINEAGE markers, wherein a LINEAGE marker is selected from the group consisting of CD2, CD3, CD10, CD14, CD16, CD31, CD45, CD64, and CD140b.

STATEMENT UNDER ARTICLE 19(1)

Support for the amendments to current claims 1-46 and 49-373 is found both in the corresponding claims as originally filed and in the specification at the descriptions of solid tumor stem cells. Applicant submits that these claims as amended are novel and inventive over the references cited in the International Search Report of September 9, 2002.

New claims 166-189 are similar to original claims 98-111, presenting similar steps in a different order. New claims 206-221 are similar to original claims 112-116, presenting similar steps in a different order. New claims 232-245 are similar to original claims 117-121, presenting similar steps in a different order. New claims 287-302 are similar to original claims 137-147, presenting similar steps in a different order.

Current claims 374-377 have been amended to recite that the Notch protein is a Notch 4 polypeptide.

Support for new claims 378-381, which recite an agent that modulates the activity of a Notch 4 ligand, is found in the specification at paragraph [59] and in EXAMPLE 12. Support for new claims 382-384 is found in the specification at paragraphs [60]-[61] and [218].

Support for new claims 385-420, which recite the killing or inhibition of solid tumor stem cells, is found in the specification at paragraph [63], in EXAMPLE 12 and in EXAMPLE 15.

Support for new claims 421-432, which recite the selective targeting of solid tumor stem cells, is found in the specification, paragraph [07], paragraph [23] and FIG. 13, paragraph [25] and FIG. 15, paragraph [26] and FIG. 16, paragraph [27] and FIG. 17, paragraph [68], paragraph [183], paragraph [193], and paragraph [199].

Support for claims 433-449, which recite the introduction of a cell dose of purified solid tumor stem cells, is found in the specification, paragraph [52] and EXAMPLE 7, TABLE 6.

Accordingly, no new matter is added.