Title: INDUCIBLE BREAST CANCER MODEL

Abstract: The present invention relates to mammals and cells comprising an expression construct for a nucleic acid encoding a breast cancer related oncogene and wherein the mammal and cell further comprise a cancer-prone genetic predisposition.
INDUCIBLE BREAST CANCER MODEL

BACKGROUND OF THE INVENTION

Breast cancer is the most common malignancy diagnosed in women in the United States and is the second leading cause of cancer mortality. As with all malignancies, breast cancer is a disease of genes. Thus, the biology of normal breast development and the mechanisms underlying the initiation, progression and metastasis of breast cancer must be understood at the molecular level to develop effective prevention and intervention strategies. For review, see, e.g., Dankort et al., Oncogene 19(8):1038-44 (2000); Baselga, Cancer Cell 2:93-5 (2002); and Moody et al., Cancer Cell 2:451-461 (2002).

SUMMARY OF THE INVENTION

This invention provides an inducible genetic model for studying the development (e.g., initiation, progression, maintenance, metastasis, regression, minimal residual disease, recurrence, and any other developmental stages) of breast cancer and identifying anti-cancer therapeutics. Featured in the invention is a mammary cell of a non-human mammal (e.g., a mouse, a rat, a hamster, a rabbit or a non-human primate) or a mammalian cell, which has a genome comprising: (a) a breast cancer related oncogene; and (b) a cancer-prone genetic predisposition, wherein, e.g., expression of the oncogene causes breast cancer in the mammal or cell proliferation, wherein the cancer regresses or cell proliferation is inhibited when, e.g., expression of the oncogene is reduced. A cancer-prone genetic predisposition may be a known cancer-prone genetic mutation. Either or both of
the breast cancer related oncogene and the cancer-prone genetic mutation may be inducible. Alternatively, the mammal comprising a breast cancer related oncogene is predisposed to developing cancer. Any animal known to have a predisposition to developing cancer may be employed, including, without limitation, A/J, C3H, C57BL/6, FVB, 129, and Balb/C strains of mice. The breast cancer related oncogene may further be operably linked to a reporter gene to facilitate detection of cells and animals having the oncogene construct.

In some embodiments, the genome of the mammal or cell of the invention comprises (a) a first expression construct comprising a nucleic acid encoding a reverse tetracycline transactivator operably linked to a mammary-specific transcriptional element; (b) a second expression construct comprising a nucleic acid encoding an oncogene operably linked to a transcriptional element that can be regulated by the reverse tetracycline transactivator and tetracycline or a tetracycline analogue; and (c) a cancer-prone genetic predisposition, wherein induced expression of the oncogene causes breast cancer in the mammal or cell proliferation, and wherein reduced expression of the oncogene results in cancer regression or inhibited cell proliferation.

In other embodiments, the genome of the mammal or cell of the invention comprises (a) a first expression construct comprising a nucleic acid encoding a Cre recombinase operably linked to a mammary-specific transcriptional element; (b) a second expression construct comprising a nucleic acid encoding an oncogene, wherein a Lox-STOP-Lox cassette is placed upstream of the transcription or translation initiation site to prevent transcription of the oncogene or translation of the oncogene's transcript; and (c) a cancer-prone genetic predisposition, wherein upon recombination between the two LoxP sites in the presence of the Cre recombinase, transcription can proceed through the nucleic acid encoding the oncogene to produce the translated oncoprotein; and wherein induced expression of the oncogene causes breast cancer in the mammal or cell proliferation; and wherein reduced expression of the oncogene results in cancer progression or inhibited cell proliferation.
In some embodiments the mammary-specific transcriptional element is a mammary-specific promoter or enhancer from, e.g., MMTV LTR, Whey acidic protein (WAP), and β-lactoglobulin (BLG).

In yet other embodiments, the genome of the mammal or cell of the invention comprises (a) a nucleic acid encoding an oncogene operably linked to a nucleic acid encoding an estrogen receptor polypeptide; and (b) a cancer-prone genetic predisposition, wherein administration of estrogen or a nonhormone analogue of estrogen to the mammal or the cell allows the correct folding of the oncogene polypeptide into a functional protein; and wherein induced expression of the oncogene causes breast cancer or cell proliferation; and wherein reduced expression of the oncogene results in cancer regression or inhibited cell proliferation. In some embodiments, the nucleic acid encoding the breast cancer related oncogene is operably linked to a nucleic acid encoding a progesterone receptor polypeptide, and where administration of progesterone or a nonhormone analogue of progesterone allows correct folding of the oncoprotein.

The breast cancer related oncogene can be, for example, Her2 (also known as neu or ErbB2), or activating mutants thereof, Bcl2, cyclin D1, myc, H-ras, K-ras, estrogen receptor gene, progesterone receptor gene, other ErbB genes (including ErbB1, ErbB3, and ErbB4), genes in the MAPK and PI3K-AKT signal transduction pathways, TGFα, PI3K, ras-GAP, Shc, Nck, src, Yes, Fyn, and viral proteins such as PyV MT and SV40 T antigens. The cancer-prone genetic mutation can be, for example, (a) a disabling mutation in a tumor suppressor gene (e.g., INK4a, P53, DCC, PTEN, Rb, DPC4, KLF6, GSTP1, ELAC2/HPC2, NKX3.1, LATS, Apaf1, Caspase 8, CHK2, BRCA1, BRCA2, Neurofibromatosis Type 1, Neurofibromatosis Type 2, Adenomatous Polyposis Coli (APC), the Wilms tumor-suppressor gene, Patches and FHIT), (b) an activating mutation in an endogenous proto-oncogene (e.g., CTNNB1, myc, ras and her2), (c) a disabling mutation in a DNA repair gene (e.g., MSH2, MSH3, MSH6, PMS2, Ku70, Ku80, DNA/PK, ATR, ATM, XRCC4 and MLH1), or (d) a disabling or activating mutation in a breast cancer related gene (e.g., Bcl2, cycline B1, myc, ras, estrogen receptor gene and Her2). The disabling mutations can be accomplished by post-transcriptional silencing using, e.g., RNAi, antisense or ribozymes.
The mammal of the invention may be transgenic, chimeric, or mosaic. The percentage of chimerism may be, e.g., at least 5%, 10%, 20%, 30%, 40% or 50%. The cell of this invention may be an ES cell, a tumor cell, a mammary cell, a tissue-specific stem cell, or a mouse cell. The cell may be derived or obtained from the mammal of this invention. Also provided are explants derived from the mammal of this invention.

This invention further provides methods of making the mammals and cells of this invention. In some embodiments, the transgenic mammal is obtained by mating mammals chimeric for the transgene. In some embodiments, a first mammal comprising a germ cell having a genome comprising a breast cancer related oncogene is mated to a second mammal comprising a germ cell having a genome comprising a cancer-prone genetic predisposition. In some embodiments, a first mammal comprising a germ cell having a genome comprising a nucleic acid encoding reverse tetracycline transactivator under the control of a mammary-specific transcriptional element is mated to a second mammal comprising a germ cell having a genome comprising an oncogene operably linked to a transcriptional element regulated by the reverse tetracycline transactivator and tetracycline or a tetracycline analogue; and wherein the germ cell of the first mammal or of the second mammal or both further comprises a cancer-prone genetic predisposition.

In some embodiments, a first mammal comprising a germ cell having a genome comprising a first expression construct comprising a nucleic acid encoding a Cre recombinase operably linked to a mammary-specific transcriptional element is mated to a second mammal comprising a germ cell having a genome comprising a second expression construct comprising a nucleic acid encoding an oncogene, wherein a Lox-STOP-Lox cassette is placed upstream of the transcription or translation initiation site to prevent transcription of the oncogene or translation of the oncogene's transcript; and wherein the germ cell of the first mammal or of the second mammal or both further comprises a cancer-prone genetic predisposition. In some embodiments, the mammary-specific transcriptional element is a mammary-specific promoter or enhancer from MMTV LTR, Whey acidic protein (WAP), and β-lactoglobulin (BLG).
In some embodiments, a first mammal comprising a germ cell having a genome comprising an expression construct comprising a nucleic acid encoding an oncogene operably linked to an estrogen receptor polypeptide is mated to a second mammal comprising a germ cell having a genome comprising a cancer-prone genetic predisposition. Alternatively, the breast cancer related oncogene is operably linked to a progesterone receptor polypeptide.

The mammal of this invention may be produced by introducing a construct comprising a breast cancer related oncogene into a zygote comprising a cancer-prone genetic predisposition; and developing the zygote into the mammal. Alternatively, the mammal may be produced by introducing a construct comprising a breast cancer related oncogene into an ES cell comprising a cancer-prone genetic predisposition; injecting the ES cell into a blastocyst or a tetraploid blastocyst; and generating the mammal.

This invention further provides methods of using the non-human mammals or the mammalian cells of this invention to identify new breast cancer related genes, surrogate biomarkers to diagnose or monitor breast cancer progression, and therapeutic agents to treat or prevent breast cancer or minimal residual breast cancer. This invention provides a method for determining whether an oncogene contributes to breast cancer maintenance, comprising the steps of: (a) providing a non-human mammal having breast cancer, wherein the mammal comprises a genome comprising: (i) the oncogene operably linked to an inducible promoter; and (ii) a cancer-prone genetic predisposition wherein the mammal comprises a mammary tumor formed in the mammal during expression of the oncogene; and (b) determining whether or not the mammary tumor regresses when expression of the oncogene is reduced, wherein mammary tumor regression is indicative of the oncogene contributing to breast cancer maintenance. Also provided is a method of identifying a breast cancer related gene, comprising the steps of: (a) establishing a first and a second molecular profile of a mammary tumor cell of the non-human mammal or mammalian cell of this invention at two different stages of breast cancer; and (b) comparing the first and second molecular profiles, wherein an alteration in expression or activity pattern of a candidate gene is indicative of the gene being a breast cancer related gene. Also provided is a
method of identifying a biomarker to diagnose or monitor breast cancer progression, comprising the steps of: (a) establishing a first molecular profile of a mammary cell of the non-human mammal of this invention or the mammalian cell of this invention wherein expression of the breast cancer related oncogene is not induced; (b) establishing a second molecular profile of the cell wherein expression of the oncogene is induced and wherein the cell becomes cancerous; and (c) comparing the first and second molecular profiles, wherein an alteration in expression or activity pattern of a candidate gene is indicative of the gene being a biomarker for breast cancer.

This invention also provides a method of identifying a therapeutic agent to treat breast cancer, comprising the steps of: (a) administering a candidate compound to the non-human mammal of this invention that has developed breast cancer; and (b) observing the effect of the compound on cancer development, wherein a decrease in tumor size, metastasis, angiogenesis or growth rate, or apoptosis of the cancer is indicative of the compound being a therapeutic agent to treat breast cancer. Alternatively, the method of identifying a therapeutic agent to prevent breast cancer, comprises the steps of: (a) administering a candidate compound to the non-human mammal of this invention that has not yet developed breast cancer; (b) inducing expression of the breast cancer related oncogene to cause cancer; and (c) observing the effect of the compound on cancer development, wherein absence of cancer formation is indicative of the compound being a therapeutic agent to prevent breast cancer. Also provided is a method of identifying a therapeutic agent to treat breast cancer, comprising the steps of: (a) contacting the cell of this invention with a candidate compound wherein the cell is a mammary tumor cell; and (b) observing the effect of the compound on cell proliferation, wherein inhibition of cell proliferation is indicative of the compound being a therapeutic agent to treat breast cancer. Also provided is a method of identifying a therapeutic agent to treat breast cancer, comprising the steps of: (a) administering a candidate compound to the non-human mammal of this invention that has developed breast cancer or to cultured breast cancer cells derived from the mammal; and (b) observing the effect of the compound on expression or activity level of a biomarker for breast cancer in the mammal or the cell, wherein an
alteration of biomarker expression or activity is indicative of the compound being a therapeutic agent to treat breast cancer. Also provided is a method of identifying a therapeutic agent to prevent breast cancer, comprising the steps of: (a) administering a candidate compound to the non-human mammal of this invention that has not yet developed breast cancer or to cultured breast cancer cells derived from the mammal; (b) inducing expression of the breast cancer related oncogene to cause cancer; and (c) observing the effect of the compound on expression or activity level of a biomarker for breast cancer in the mammal or the cells, wherein absence of alteration of biomarker expression or activity is indicative of the compound being a therapeutic agent to treat breast cancer. Also provided is a method of identifying a therapeutic agent to treat breast cancer, comprising the steps of: (a) contacting the cell of this invention with a candidate compound wherein the cell is a mammary tumor cell; and (b) observing the effect of the compound on expression or activity level of a biomarker for breast cancer in the cells, wherein an alteration of biomarker expression or activity is indicative of the compound being a therapeutic agent to treat breast cancer. Also provided is a method of identifying a therapeutic agent to prevent breast cancer, comprising the steps of: (a) establishing a first molecular profile of a non-cancerous mammary cell of the non-human mammal of this invention, or a mammary cell derived from the mammal, by identifying a plurality of biomarkers whose patterns of expression or biological activity correspond to the non-cancerous stage of the mammary cell, and wherein expression of the breast cancer related oncogene is not induced; (b) contacting the mammary cell with a candidate compound; (c) establishing a second molecular profile of the contacted mammary cell, wherein the second pattern of expression or biological activity of the biomarkers correspond to the mammary cell, wherein expression of the oncogene is induced to cause cancer; and (d) comparing the first and second profiles, wherein substantial similarity of the first and second profiles is indicative of the compound being a therapeutic agent to prevent breast cancer.

This invention also provides a method of identifying a gene involved in minimal residual breast cancer, comprising the steps of: (a) establishing a first molecular profile for a non-cancerous mammary cell of the non-
human mammal of this invention; and (b) establishing a second molecular profile
for a mammary cell of the non-human mammal of this invention having minimal
residual breast cancer; (c) establishing a third molecular profile for a cancerous
mammary cell of the non-human mammal of this invention wherein cancer is
induced by expression of the breast cancer related oncogene; and (d) comparing
the first, second and third profiles, wherein an alteration in expression patterns of
the gene in the first and second profiles while substantial similarity of expression
patterns of the gene in the second and third profiles is indicative of the gene being
involved in minimal residual breast cancer. Also provided is a method of
identifying a therapeutic agent to treat or prevent minimal residual breast cancer,
comprising the steps of: (a) administering a candidate compound to the non-human
mammal of this invention, wherein the mammal has minimal residual breast
cancer; and (b) observing the effect of the compound on expression or activity
level of a gene involved in minimal residual breast cancer, wherein an alteration in
expression or activity level of the gene is indicative of the compound being a
therapeutic agent to treat or prevent minimal residual breast cancer. The profiles
can be established by any standard technique, e.g., suppression subtraction,
differential display, proteomic analysis, serial analysis of gene expression and
comparative genomic hybridization.

Other features and advantages of the invention are described in the
following detailed description and drawings.

**BRIEF DESCRIPTION OF THE DRAWINGS**

Fig. 1 is a drawing illustrating an expression construct of a reverse
tetracycline transactivator (rtTA) under the control of a MMTV LTR promoter.

"Intron 1" denotes a sequence from mouse C14C heavy chain immunoglobulin
variable region intron (Rothenstuhl et al., PNAS 91:126163-12167 (1994)). "Intron
2" denotes a sequence spanning 6062-6247 bp of Adenovirus Type 5 (van Beveren
et al., Gene 16:179-189 (1981)). "rtTA-M2" denotes a rtTA mutant (Urlinger et

Fig. 2 is a drawing illustrating an expression construct of a Her2
oncogene whose expression is inducible by a reverse tetracycline transactivator
and tetracycline. “TetO” denotes a Tet operator sequence linked to a minimum Pol
II promoter. "Her2" denotes a Her2 oncogene or an activating mutant thereof. "SV40 pA" denotes a polyA sequence from a SV40 virus.

Fig. 3A is a graph showing the results of assaying luciferase activity in mammary cells isolated from mouse chimera 260 and cultured in the presence or absence of doxycycline. Fig. 3B is a graph showing the results of Her2 expression in the same cells using RT-PCR.

Fig. 4A and 4B are graphs showing the results of tumor regression studies in two different chimeric mice. In Fig. 4A, doxycycline was withdrawn at day 0. In Fig 4B, doxycycline was withdrawn at day 7.

**DETAILED DESCRIPTION OF THE INVENTION**

This invention features a nonhuman mammal (e.g., a mouse, a rat, a hamster, a rabbit or a non-human primate), in which the genome of at least some of its cells contains a constitutive or inducible system for expressing an oncogene (e.g., a nucleotide sequence encoding an oncoprotein) that is related to breast cancer. In some embodiments, the genome of the cells further contains a genetic predisposition that renders the mammal even more susceptible to cancer than it would otherwise be; in these embodiments, the mammal develops spontaneous breast cancer much sooner than a mammal that does not contain the genetic predisposition. In one embodiment, upon induced expression of the oncogene in the mammary gland, the mammal develops spontaneous breast hyperplasia that may progress to tumors (e.g., epithelial tumors and/or neuroendocrine tumors) and/or develops breast cancer e.g., stages I-IV of breast cancer including hyperplasia, lobular carcinoma in situ (LCIS), ductal carcinoma in situ (DCIS), invasive breast carcinoma, invasive breast carcinoma that has spread to lymph nodes). In some embodiment, the genetic predisposition is one or more genetic mutation such as disabling mutations in a tumor suppressor gene, a disabling mutation in a breast cancer related gene, a disabling mutation in a DNA repair gene, or an activating mutation in an endogenous proto-oncogene. The genetic mutation may also be controlled by an inducible system. In some embodiment, the breast cancer related oncogene is constitutively expressed and the genetic mutation that renders the mammal more susceptible to developing cancer is inducible, wherein induction of the mutation results in development of breast cancer (e.g.,
stages I-IV of breast cancer including hyperplasia, lobular carcinoma in situ (LCIS), ductal carcinoma in situ (DCIS), invasive breast carcinoma, invasive breast carcinoma that has spread to lymph nodes).

Tumors may develop within 1, 3, or 5 days; or 1, 2, 4, 6, or 8 weeks after induction of expression of the oncogene. The tumors may show the pathology of invasive carcinoma or of any other stage of breast cancer. The animals of the invention may contain 1 or more, e.g., 2, 3, or 4 tumors per mammary gland. The tumors may be estrogen-dependent or estrogen-independent. Metastasis may eventually occur. Induction of tumorigenicity can be determined, for example, by monitoring the mammal for development of a tumor. Alternatively, a soft agar assay or any of the other assays described herein can be employed. The tumors may regress when expression of the oncogene is turned off or reduced. Regression can begin after 0-5 days after the oncogene is turned off or reduced. The timing of regression may depend on the half-life of the oncoprotein and/or the half-life of the inducing reagent.

The mammal of this invention can be a transgenic animal all of whose germ and somatic cells contain the inducible oncogene and the genetic predisposition. Alternatively, the mammal is a chimeric or mosaic animal in which only some of its somatic and/or germ cells contain the oncogene and the genetic predisposition. For example, the percentage of chimerism is at least 5%, 10%, 20%, 30%, 40% or 50%.

The inducible breast cancer model of this invention provides an advantageous way to study breast cancer as compared to the conventional tumor explant models. In this model, breast cancer occurs only upon expression of the introduced oncogene in the animal host. Further, a breast cancer related oncogene may be repeatedly inducible, reducible and re-inducible. Thus, each host represents a distinct tumorigenesis event. In a traditional tumor explant model, however, each tumor originates from implanted cells of the same tumor cell line. Thus, the inducible model better resembles clinical development of breast cancer, where each patient and/or each tumor represents a distinct tumorigenic event. Further, tumors in this model arise de novo in a natural mammary environment and their development hence better resembles clinical conditions.
1. INDUCIBLE ONCOGENES

Breast cancer related oncogenes useful in this invention include, without limitation, *Her2* (also known as *neu* or *ErbB2*), or activating mutants thereof, *Bcl2, cyclin D1, myc, H-ras, K-ras*, estrogen receptor gene, progesterone receptor gene, other ErbB genes (including *ErbB1, ErbB3*, and *ErbB4*), genes in the MAPK and PI3K-AKT signal transduction pathways, *TGFα, PI3K, ras-GAP, Shc, Nick, src, Yes, Fyn, β-catenin*, and viral proteins such as PyV MT and SV40 T antigens. See, e.g., Baselga, *supra*; Dankort et al., *supra*. Expression of the oncogene is induced by any inducible transcription system, e.g., the Cre-Lox systems and any of the inducible transcription systems for RNA polymerase II (e.g., the tetracycline transactivator systems, reverse tetracycline transactivator systems, ecdysone systems, methallothioneine systems, *LacO/IPTG* systems, and *TetO/tetracycline* systems). Inducible transcription systems for RNA polymerases I and III (e.g., using the U6, H1, 5S or 7SK promoter) also can be used with or without modifications.

In an exemplary Cre-Lox system, a Lox-STOP-Lox cassette is placed upstream to the transcription or translation initiation site of a transgene that is an oncogene, preventing transcription of the gene or translation of the gene’s transcript. The genome of the host cell also contains the coding sequence for a Cre recombinase under the transcriptional control of one or more mammary-specific elements (e.g., promoters and/or enhancers). The mammary-specific element may be stage-specific. Useful mammary-specific transcription control elements may be those from genes specifically or preferentially expressed in, e.g., mammary epithelial cells or terminally differentiated mammary epithelial cells, including, without limitation, the MMTV LTR, Whey acidic protein (WAP), and β-lactoglobulin (BLG). In some embodiments, a mammary-specific enhancer may be used in combination with a minimal promoter. In some embodiments, a portion of the mammary-specific promoter sufficient to direct expression in mammary tissue may be used. The Cre recombinase is thus specifically or preferentially expressed in the mammary gland, causing deletion of the STOP signal flanked by the Lox sites and allowing expression of the oncogene. In some embodiments, the
promoter for the oncogene is an inducible promoter such that expression of the oncogene in the mammary gland is inducible.

In an exemplary reverse tetracycline transactivator system, the oncogene is operably linked to a promoter activatable by a reverse tetracycline transactivator and tetracycline (or an analogue thereof such as doxycycline). Typically, this promoter contains a Tet operator sequence. The genome of the host cell also contains a reverse tetracycline transactivator transgene regulated by mammary-specific transcription control elements as described above. Expression of the oncogene can thus be turned on (i.e., induced) or off (i.e., non-induced) specifically in the mammary gland upon administration or withdrawal of tetracycline or its analogue. See also Moody, supra.

Expression of the oncogene may also be inducibly switched on or off by fusing the oncogene to, e.g., a coding sequence for an estrogen receptor polypeptide sequence, where administration of estrogen or a nonhormone estrogen analogue (e.g., hydroxytamoxifen) will allow the correct folding of the oncogene polypeptide into an functional protein. See, e.g., Moody, supra. A similar inducible system involves fusion of the oncogene with a coding sequence for a progesterone receptor polypeptide sequence wherein expression of the oncogene is induced by progesterone or a nonhormone progesterone analogue (e.g., RU486).

Various vectors can be used for the oncogene expression. These vectors can be based on plasmids, transposons or viruses such as retroviruses, adenoviruses, and lentiviruses. The vectors can be introduced into zygotes, embryonic stem (ES) cells, tissue-specific stem cells, organ explants or the mammary gland in situ as required via a variety of methods, including but not limited to, liposome fusion (transposomes), routine nucleic acid transfection methods such as electroporation, calcium phosphate precipitation, and microinjection, and infection by viral vectors.

A reporter gene may be fused to the oncogene. Such a reporter gene can be, for example, a fluorescent protein such as a green fluorescent protein, a yellow fluorescent protein, a blue fluorescent protein, a red fluorescent protein (e.g., dsRed), or any variation thereof; or a luminescent protein such as luciferase and β-galactosidase.
2. CANCER-PRONE GENETIC PREDISPOSITIONS

In addition to an above-described inducible oncogene, the mammal of the invention may further comprise one or more genetic predispositions rendering it even more susceptible to tumorigenesis. Animals with such genetic predispositions include, without limitations, tumor-prone mouse strains (e.g., A/J, C3H, C57BL/6, FVB, 129 and Balb/C). The genetic predispositions may be due to one or more genetic mutations, including, without limitations, disabling (e.g., null, conditionally null, or dominant negative) mutations in a tumor suppressor gene (e.g., INK4a, P53, DCC, PTEN, Rb, DPC4, KLF6, GSTP1, ELAC2/HPC2, NKX3.1, LATS, Apaf1, Caspase 8, CHK2, BRCA1, BRCA2, Neurofibromatosis Type 1, Neurofibromatosis Type 2, Adenomatous Polyposis Coli (APC), the Wilms tumor-suppressor gene, Patches or FHIT), disabling or activating mutations in certain breast cancer related genes (e.g., Bcl2, cyclin D1, myc, ras, estrogen receptor gene, or Her2), disabling mutations in a DNA repair gene (e.g., MSH2, MSH3, MSH6, PMS2, Ku70, Ku80, DNA/PK, ATR, ATM, XRCC4, or MLH1), and activating mutations in an endogenous proto-oncogene (e.g., CTNNB1, myc, and ras). See, e.g., Jacks et al., Nature 359:295-300 (1992); Donehower et al., Nature 356:215-21 (1992); Serrano et al., Cell 85:27-37 (1996); and Hakem et al., Annu. Rev. Genet. 35:209-41 (2001). These mutations can be introduced into the genome of a host cell by well established homologous recombination technologies (e.g., gene knock out or knock in) or by introduction of transgenes.

In some embodiments, the disabling mutations are accomplished by post-transcriptional silencing using, e.g., RNA interference (RNAi), antisense or ribozymes. For example, RNAi constructs may be introduced into the host genome to inhibit expression of the target gene (e.g., the tumor suppressor gene, breast cancer related gene, or DNA repair gene). RNAi is a sequence-specific posttranscriptional gene silencing mechanism triggered by double-stranded RNA (dsRNA). It causes degradation of mRNAs homologous in sequence to the dsRNA. See, e.g., Elbashir et al., Methods 26:199-213 (2002); McManus and Sharp, Nature Reviews 3:737-747 (2002); Hannon, Nature 418:244-251 (2002); Brummelkamp et al., Science 296:550-553 (2002); Tuschi, Nature Biotechnology 20:446-448 (2002); Czauderna, Nucleic Acids Res. 21(31):127 (2003); U.S. Patent
6,506,559; U.S. Application US2002/0086356 A1; WO 99/32619; WO 01/36646; and WO 01/68836. With the use of RNAi constructs, one can further control tumorigenesis in the animal by inducibly expressing RNAi molecules that interrupt activity of the targeted gene. Similarly, antisense or ribozyme constructs, wherein the antisense or ribozyme is inducibly expressed, can be used to inducibly target expression, function or activity of a targeted gene.

Alternatively, the cancer-prone mutations may be inducible while the breast cancer related oncogene is or is not inducible.

3. MAMMALS OF THE INVENTION

A variety of approaches can be used to generate the mammals of this invention. Under one approach, transgenic, chimeric, or mosaic mammals harboring oncogene expression constructs are inter-crossed with mammals having a cancer-prone genetic predisposition described above, to generate a mammal predisposed to developing cancer and having the inducible oncogene. For instance, two mammal lines are prepared for mating, where one line contains a reverse tetracycline transactivator gene under the control of a mammary-specific promoter (e.g., the MMTV-rtTA construct shown in Fig. 1), and the other line harbors an oncogene linked to a promoter containing a tetO sequence (e.g., the tetO-Her2 construct shown in Fig. 2). Before mating, each of these two lines may be intercrossed with INK4a+/- or INK4a-/- mammals to generate transgenic, chimeric or mosaic mammals whose genome contains the oncogene under the inducible control of tetracycline in the mammary gland (e.g., containing both MMTV-rtTA and tetO-Her2) in a heterozygous or homozygous INK4a null background. Alternatively, each line may be intercrossed with mammals with a heterozygous or homozygous null mutation in another tumor suppressor gene (e.g., P53 or Rb). See, e.g., Donehower et al., supra; U.S. Patent 5,919,997; WO 01/09308; Chin et al., Genes Devel. 11:2822-34 (1997); and Jacks et al., Nature 359:295-300 (1992).

Under another approach, constructs for the inducible oncogene are introduced into zygotes derived from animals already containing a cancer-prone genetic predisposition. For example, the constructs are injected into the two-cell stage of an embryo or injected into a zygote. The embryo or zygote can then be
developed into a transgenic or mosaic animal. Alternatively, the constructs are stably integrated into ES cell lines derived from animals containing the genetic predisposition. The ES cells are then injected to blastocysts to generate chimeric or mosaic animals containing the oncogene expression constructs in the background of the genetic predisposition. These ES cells can also be injected into tetraploid blastocysts to generate transgenic animals whose genome contains the oncogene expression constructs in the background of the genetic predisposition. When chimeric and mosaic mammals are used, it is desirable to determine whether their mammary glands contain the oncogene transgene. To better identify these mammals, one can incorporate a reporter gene into the oncogene construct.

The mammals of this invention may develop breast cancer (e.g., stages I-IV of breast cancer including hyperplasia, lobular carcinoma in situ (LCIS), ductal carcinoma in situ (DCIS), invasive breast carcinoma, invasive breast carcinoma that has spread to lymph nodes) spontaneously within a few months of the induction of oncogene expression. The mammals may be treated with carcinogens (e.g., 9,10-dimethyl-1,2-benzanthracene, ENU, urethane, Dimethylhydrazine and Azoxyethane) to expedite this process. Mammary tumor explants may be obtained from the mammals of this invention.

4. MAMMALIAN CELLS OF THE INVENTION

Mammalian cells of this invention comprise an oncogene and a cancer-prone genetic predisposition where induced expression of the oncogene or of the genetic predisposition causes the cell to become cancerous or tumorigenic where cancer or tumorigenesis is inhibited when expression of the oncogene or genetic predisposition is reduced. Tumorigenesis or cancer development may be assayed using standard techniques, e.g., by assaying cell proliferation, invasive capability, immortalization, anchorage independence. These properties may be determined using any method including those described herein.

The cells may be obtained or derived from the mammals of the invention. In some embodiments, the mammalian cells are ES cells, tumor cells, tissue-specific stem cells, or mammary cells.
5. **EXEMPLARY USES**

The mammals of this invention and mammary cells derived from the mammals can be used to delineate the initiation, progression, maintenance, regression, minimal residual disease, recurrence, or any other developmental stages of breast cancer. They can also be used to develop and validate anti-cancer therapeutics. The following describes a few such uses.

(a) **Identification of new tumor related genes**

The mammals or cells of this invention may be used to identify new breast cancer related genes, e.g., breast cancer suppressor genes or a gene suspected of being required for tumor initiation, progression, maintenance, metastasis, regression, minimal residual disease, recurrence, and/or any other developmental stages. For example, a mammal may be obtained as having an expression construct comprising a candidate oncogene, which is operably linked to an inducible promoter so that expression of the oncogene can be repeatedly inducible, reducible and re-inducible, and a cancer-prone genetic predisposition. Then, if the breast cancer regresses when expression of the oncogene is reduced, the oncogene is involved in breast cancer development or maintenance.

Forward genetic screens may also be used to identify new breast cancer related genes. The genetic screens can be conducted using, e.g., retroviral insertion, transposon insertion, genetrap vectors, RNAi or inducible RNAi. Using these elements allows for identification of new breast cancer related genes as target genes. Genetic screens are described in, e.g., US Patent Application No. 2003003478; Mikkers et al., *Adv. Cancer Res.* 88:53-99 (2003); and Suzuki et al., *Nat. Genet.* 32(1):166-74 (2002).

In another example, a gene expression profile for breast cancer undergoing different stages (e.g., genesis, maintenance, progression, regression or recurrence) due to expression or nonexpression of the introduced oncogene can be established. Then, comparisons of expression profiles at different stages of cancer development can be performed to identify genes whose expression patterns are altered. Such genes may be breast cancer related genes. For example, tumor initiation genes might be turned off during tumor maintenance. Approaches that focus on genes and pathways involved in the tumor maintenance, rather than initial
tumor development, may lead to the development of better anti-cancer therapies and diagnosis for advanced disease. Alternatively, genes identified as being involved in initiation of cancer can be used in the discovery of therapies and diagnosis relating to preventive or early control of the disease.

Techniques used to establish gene expression profiles include the use of, e.g., suppression subtraction (in cell culture), differential display, proteomic analysis, serial analysis of gene expression (SAGE) and comparative genomic hybridization (CGH). To allow high throughput profiling, cDNA and/or oligonucleotide microarrays can be used.

(b) Identification of surrogate biomarkers

The mammals of the invention may also be used to identify surrogate biomarkers for diagnosis or to follow breast cancer progression in a mammal (e.g., a mouse, a rat, a rabbit, a nonhuman primate, or a human). The biomarkers can be identified based on the differences between expression profiles of the induced (i.e., expression of a breast cancer related oncogene is induced) and non-induced (i.e., expression of the oncogene is not induced) stages (e.g., genesis, maintenance, progression, regression and recurrence) in non-human mammals or mammalian cells of this invention. Blood, urine or other body fluids from the mammal or the cells can be tested with ELISAs or other assays to determine which biomarkers are released from the diseased mammary tissue into circulation during genesis, maintenance, progression or regression of the cancer. Such diagnosis may involve detecting expression or activity level of the biomarker, wherein an abnormally high or low level relative to control (e.g., at least about 50%, 100%, 150%, 200%, 250%, or 300% higher or at least 10%, 25%, 50%, 75%, or 100% lower) is indicative of an abnormal condition. These biomarkers are particularly useful clinically in detecting or monitoring breast cancer progression post cancer therapy. These biomarkers can also be used clinically to assess the toxicity of any breast cancer therapy.

(c) Identification of therapeutic agents

The mammals of the invention may further be used to screen therapeutic agents for breast cancer. One such method involves administering a candidate compound to a mammal that has developed breast cancer or contacting
breast cancer cells derived from such a mammal with a candidate compound. Then one can observe the effect of the compound on cancer regression, as indicated by, e.g., reduction of tumor size, metastasis, angiogenesis and growth rates, or apoptosis or inhibited proliferation of the cultured cancer cells. Several methods may be employed to evaluate the effect of the compound.

In an exemplary method of determining cell proliferation, growth of cells is examined in media containing 10%, 2.5%, and 0.5% serum. Growth curves over 10 to 14 day periods can be analyzed by cell counts on days 0, 1, 3, 5, 7, 9, 11, and 13. Quantitative measure of S phase progression can be determined by BrdU incorporation. These two assays provide both static and dynamic views of the proliferative history of these cells. For example, if the cell culture has a higher S phase percentage than the Cre-excised control, as measured by BrdU incorporation, and yet their growth curves are overlapping, this suggests that, although there is increased S phase progression, there must be increased death, resulting in similar growth curves. To determine the rate of apoptosis in low and high serum conditions, Annexin V staining by FACS can be performed. Alternatively, cells can be seeded in chamber slides and fixed in methanol: acetone for TUNEL staining.

In an exemplary method to assay invasive capability, Boyden chamber assays can be performed to measure the migration of cells (Shimizu et al., Biochem. Biophys. Res. Comm. 264:751, 1999). Briefly, the lower well of a chamber is filled with 600 Tl of medium with 10% or 2.5% FCS, and the upper well is seeded with 400 Tl of cell suspension. A cellulose acetate membrane filter is then interposed between the two chambers. The chambers are kept in a humidified atmosphere of 5% CO₂ at 37°C for 4 hours. Filters are then washed, fixed with methanol: acetone, and stained with crystal violet. The number of cells that migrate into the filter and reach its lower side can be determined microscopically. Triplicate assays can be performed for each cell line and its controls.

In an exemplary method to determine immortalization, a low-density seeding assay can be used as a surrogate assay for immortalization potential. In this assay, 2500 cells are seeded per well in a 6 well plate. Cells with
high potential for immortalization are able to grow to form visible colonies in 14 days. The number of emerging colonies can be used as a quantitative measure for the immortalization potential of those cells.

In an exemplary method to assay tumorigenecity in explants, anchorage independence is evaluated in soft-agar assays. For example, in soft-agar, 10,000 cells are seeded per well in a 6-well plate. Colony formation is monitored daily by microscopic inspection. Cell clusters of greater than 0.5 mm in size are counted as a colony. The number of colonies is a quantitative marker for the tumorigenic potential of the cells.

Alternatively, one can observe the effect of the compound on expression or activity level of a biomarker for the breast cancer in a mammal or cell of the invention, where a normalizing change of this level is indicative of the effectiveness of the compound. In another embodiment, a candidate compound is administered to a mammal or cell of the invention wherein the inducible oncogene is expressed to cause formation of cancer. If no cancer results, the compound is a candidate prophylactic agent capable of preventing tumor formation and/or growth.

In other embodiments, a therapeutic agent can be identified based on the molecular profile it elicits. To do this, a first molecular profile (e.g., transcriptional, proteomic or genomic) of the mammary cells from the mammals of this invention or the mammalian cells of this invention is established by, e.g., identifying a plurality of biomarkers whose patterns of expression or biological function alternations correspond to the non-induced (i.e., expression of a breast cancer related oncogene is not induced) stage (e.g., genesis, maintenance, progression, regression and recurrence) of the mammal or the mammalian cells. A second molecular profile of these biomarkers is established corresponding to the induced (i.e., expression of the breast cancer related oncogene is induced in the mammal or the mammalian cells) stage (e.g., genesis, maintenance, progression, regression and recurrence) of the mammal or the mammalian cells in the presence of a candidate compound. The two profiles are compared, wherein substantial similarity of the two profiles indicates that the test compound is a potential anti-cancer drug. “Substantial similarity” means that the Pearson correlation coefficient of biomarker expression/activity for the two molecular profiles is statistically
significant, with a $p$ value of less than 0.1 (e.g., less than 0.05, 0.02, or 0.01). The non-overlapping portion between the two profiles may represent nonspecific activity of the candidate compound and allow prediction of the potential toxicity of the compound.

(d) **Study of minimal residual diseases**

The mammals of this invention may be used to study minimal residual breast cancer and to identify therapeutic agents to treat minimal residual breast cancer. For example, a non-human mammal having a genome, which comprises an inducible breast cancer related oncogene and a cancer-prone genetic predisposition, has minimal residual diseases if tumor recurrence occurs at a site of a previous tumor, which formed when expression of the oncogene was induced, and regressed when expression of the oncogene was reduced.

Using the mammals, one can establish molecular profiles I, II and III for mammary cells derived from a mammal having breast cancer (I), having minimal residual breast cancer (II) or having neither breast cancer nor minimal residual breast cancer (III), respectively. One can then compare these molecular profiles to identify genes whose expression patterns or activities are altered. Genes are identified as being involved in minimal residual breast cancer if substantial similarity exists between their expression in profiles I and II, but an alteration exists between profiles II and III.

Therapeutic agents may be identified by administering a candidate compound to a mammal having residual breast cancer. An alteration in expression or activity of a gene involved in minimal residual breast cancer indicates that the compound may be useful as a therapeutic agent to treat minimal residual breast cancer. In another embodiment, compounds can be identified as being useful for preventing minimal residual breast cancer.

6. **Examples**

The following describes several methods of making and using the inducible breast cancer model. These examples are intended to illustrate the methods and materials of the present invention. Suitable modifications and adaptations of the described conditions and parameters normally encountered in the art which are obvious to those skilled in the art are within the spirit and scope
of the present invention. These examples are not to be construed as limiting the scope of the invention in any way.

**Example 1**

Transgenic mice having the constructs shown in Figs. 1 and 2 are established and bred with transgenic mice having an INK4a +/- mutation. The resultant mice contain a rtTA inducible system for mammary Her2 expression in an INK4a +/- background. These mice are fed with doxycycline-containing drinking water (2 mg/ml sucrose water) or food pellets and observed for spontaneous tumor development in the mammary gland. Primary tumors are adapted to culture by mechanical mincing with sterilized razor blades and brief trypsinization and maintained on RPMI media containing 10% serum and supplemented with doxycycline (2 µg/ml media).

To perform histological analysis of the mammary gland, mammary tissue samples or derivative cells are fixed and embedded. An antibody against a breast cancer related protein, including, without limitation, antibodies to the ErbB2 receptor such as 2C4, and Pan-Cytokeratin AE1/AE3 antibody, is used to demonstrate the distribution of such protein in the tumor tissue samples or derivative tumor cells at different stages of tumor development (e.g., progression and regression). See, e.g., Baselga, supra; and Kohlberger et al., Anticancer Res. 21(1B):697-698 (2001).

To generate growth curves of cells from derivative breast cancer cell lines, the cells are seeded at a density of 20,000 cells per well in a 12-well plate in media with or without doxycycline. Media is changed every 3 days for all samples. Duplicate wells are trypsinized, and cell numbers are counted by hemacytometer at different time points and plotted against time. Studies are conducted in media containing 10%, 1% and 0.5% serum. Growth curve determinations are performed in cells maintained on doxycycline prior to experiments as well as cells already removed from doxycycline for 3 days.

**Example 2**

Mice comprising the following constructs MMTV-rtTA (reverse tetracycline transactivator (rtTA) under the control of a MMTV LTR promoter),
and either TetO-Her2<sup>V664E</sup> (A mutant Her2 under the control of a Tet operator sequence, wherein the valine at amino acid residue 664 is substituted with glutamine) or TetO-K-ras<sup>G12V</sup> (A mutant K-ras under the control of a Tet operator sequence, wherein the glycine at amino acid residue 12 is substituted with valine) are generated in an Ink4a homozygous null background. Optionally, the founder mice are bred to produce transgenic mice.

The inducibility of the oncogenes is analyzed by northern blot analysis or RT-PCR. Briefly, mammary glands are removed from chimeric mice and digested with collagenase. Half of the organoids collected are cultured in the presence of doxycyclin and the other half in the absence of doxycycline for 5 days. Cells are trypsinized at the end of the culturing period and used for RNA extraction for RT-PCR.

To induce spontaneous tumor development, the mice produced above are fed 2mg/ml doxycycline through drinking water. Tumors that arise are measured daily with a caliper.

Tumor regression studies are carried out by removing doxycycline from the drinking water and daily measuring tumor size using a caliper.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Exemplary methods and materials are described below, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention. All publications and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control. The materials, methods, and examples are illustrative only and not intended to be limiting. Throughout this specification and claims, the word “comprise,” or variations such as “comprises” or “comprising” will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.
What is Claimed is:

1. A non-human mammal, wherein a mammary cell of said mammal comprises a genome comprising:
   (a) a breast cancer related oncogene; and
   (b) a cancer-prone genetic predisposition,
   wherein expression of said oncogene causes breast cancer in said mammal, and wherein said cancer regresses when expression of said oncogene is reduced.

2. A non-human mammal wherein the mammal is predisposed to develop cancer and wherein a mammary cell of said mammal comprises a genome comprising an inducible breast cancer related oncogene.

3. The non-human mammal according to claim 2, wherein the mammal is a mouse selected from the group consisting of A/J, C3H, C57BL/6, FVB, 129 and Balb/C.

4. The non-human mammal according to claim 1, wherein a reporter gene is operably linked to said oncogene.

5. A non-human mammal, wherein a mammary cell of said mammal comprises a genome comprising:
   (a) a first expression construct comprising a nucleic acid encoding a reverse tetracycline transactivator operably linked to a mammary-specific transcriptional element;
   (b) a second expression construct comprising a nucleic acid encoding an oncogene operably linked to a transcriptional element that can be regulated by said reverse tetracycline transactivator and tetracycline or a tetracycline analogue; and
   (c) a cancer-prone genetic predisposition,
   wherein induced expression of said oncogene causes breast cancer in said mammal; and wherein reduced expression of said oncogene results in cancer progression.

6. A non-human mammal, wherein a mammary cell of said mammal comprises a genome comprising:
(a) a first expression construct comprising a nucleic acid encoding a Cre recombinase operably linked to a mammary-specific transcriptional element;

(b) a second expression construct comprising a nucleic acid encoding an oncogene, wherein a Lox-STOP-Lox cassette is placed upstream of the transcription or translation initiation site to prevent transcription of the oncogene or translation of the oncogene's transcript; and

(c) a cancer-prone genetic predisposition,

wherein upon recombination between said two LoxP sites in the presence of the Cre recombinase, transcription can proceed through said nucleic acid encoding said oncogene to produce the translated oncoprotein; and wherein induced expression of said oncogene causes breast cancer in said mammal.

7. The non-human mammal according to claim 5 or 6, wherein said mammary-specific transcriptional element is a mammary-specific promoter or enhancer.

8. The non-human mammal according to claim 7, wherein said mammary-specific promoter or enhancer is derived from a gene selected from the group consisting of MMTV LTR, Whey acidic protein, and β-lactoglobulin.

9. A non-human mammal, wherein a mammary cell of said mammal comprises a genome comprising:

(a) a nucleic acid encoding an oncogene operably linked to a nucleic acid encoding an estrogen receptor polypeptide; and

(b) a cancer-prone genetic predisposition,

wherein administration of estrogen or a nonhormone estrogen analogue to said mammal allows the correct folding of the oncogene polypeptide into a functional protein; and wherein induced expression of said oncogene causes breast cancer in said mammal; and wherein reduced expression of said oncogene results in cancer regression.

10. A non-human mammal, wherein a mammary cell of said mammal comprises a genome comprising:
(a) a nucleic acid encoding an oncogene operably linked to a nucleic acid encoding a progesterone receptor polypeptide; and
(b) a cancer-prone genetic predisposition,
wherein administration of progesterone or a nonhormone progesterone analogue to said mammal allows the correct folding of the oncogene polypeptide into a functional protein; and wherein induced expression of said oncogene causes breast cancer in said mammal; and wherein reduced expression of said oncogene results in cancer regression.

11. The non-human mammal according to any one of claims 1 to 10 wherein said cancer-prone genetic predisposition is a cancer-prone genetic mutation.

12. The non-human mammal according to claim 11, wherein the oncogene or the cancer-prone genetic mutation or both the oncogene and the cancer-prone genetic mutation is inducible.

13. The non-human mammal according to claim 11, wherein said cancer-prone genetic mutation is selected from the group consisting of (1) a disabling mutation in a tumor suppressor gene, (2) an activating mutation in an endogenous proto-oncogene, (3) a disabling mutation in a DNA repair gene and (4) a disabling or activating mutation in a breast cancer related gene.

14. The non-human mammal according to claim 13, wherein said disabling mutation is accomplished by post-transcriptional silencing.

15. The non-human mammal according to claim 14, wherein said post-transcriptional silencing is accomplished by RNA interference, antisense or ribozyme.

16. The non-human mammal according to any one of claims 1 to 15, wherein said oncogene is selected from the group consisting of Her2, an activating variant of Her2, Bcl2, cyclin D1, myc, H-ras, K-ras, estrogen receptor gene, progesterone receptor gene, a non-Her2 ErbB gene, a gene in the MAPK signal transduction pathway, a gene in the PI3K-AKT signal transduction pathway, TGFe, PI3K, ras-GAP, Shc, Nck, src, Yes, Fyn, β-catenin, and a viral protein.
17. The non-human mammal according to claim 16, wherein said non-

*Her2* ErbB gene is selected from the group consisting of *ErbB1, ErbB3*

and *ErbB4*.

18. The non-human mammal according to claim 16, wherein said viral protein is SV40 T or PyMT.

19. The non-human mammal according to any one of claims 13 to 18, wherein said tumor suppressor gene is selected from the group consisting of INK4a, P53, DCC, PTEN, Rb, DPC4, KLF6, GSTP1, ELAC2/HPC2, NKX3.1,

LATS, Apaf1, Caspase 8, CHK2, BRCA1, BRCA2, Neurofibromatosis Type 1, Neurofibromatosis Type 2, Adenomatous Polyposis Coli (APC), the Wilms tumor-suppressor gene, Patches and FHIT.

20. The non-human mammal according to any one of claims 13 to 19, wherein the proto-oncogene is selected from the group consisting of CTNNB1, *myc*, and *ras*.

21. The non-human mammal according to any one of claims 13 to 20, wherein the DNA repair gene is selected from the group consisting of MSH2, MSH3, MSH6, PMS2, Ku70, Ku80, DNA/PK, ATR, ATM, XRCC4 and MLH1.

22. The non-human mammal according to any one of claims 13 to 21, wherein the breast cancer related gene is selected from the group consisting of *Bcl2*, cycline B1, *myc, ras*, estrogen receptor gene and *Her2*.

23. The non-human mammal according to any one of claims 1 to 22, wherein said mammal is transgenic.

24. The non-human mammal according to any one of claims 1 to 22, wherein said mammal is chimeric.

25. The non-human mammal according to claim 24, wherein at least a germ cell comprises said oncogene and said genetic predisposition.

26. The non-human mammal according to claim 24, wherein said percentage of chimerism is at least 5%, 10%, 20%, 30%, 40% or 50%.

27. The non-human mammal according to any one of claims 1 to 26, wherein said mammal is a mouse.
28. A mammalian cell, wherein said cell comprises a genome comprising:
   (a) a breast cancer related oncogene; and
   (b) a cancer-prone genetic predisposition,
   wherein expression of said oncogene causes cell proliferation, and wherein said proliferation is inhibited when expression of said oncogene is reduced.

29. The cell according to claim 28, wherein a reporter gene is operably linked to said oncogene.

30. A mammalian cell, wherein said cell comprises a genome comprising:
   (a) a first expression construct comprising a nucleic acid encoding a reverse tetracycline transactivator operably linked to a mammary-specific transcriptional element; and
   (b) a second expression construct comprising a nucleic acid encoding an oncogene operably linked to a transcription control element regulated by said reverse tetracycline transactivator and tetracycline or a tetracycline analogue; and
   (c) a cancer-prone genetic predisposition,
   wherein induced expression of said oncogene causes cell proliferation; and wherein said proliferation is inhibited when expression of said oncogene is reduced.

31. A mammalian cell, wherein said cell comprises a genome comprising:
   (a) a first expression construct comprising a nucleic acid encoding a Cre recombinase operably linked to a mammary-specific transcriptional element; and
   (b) a second expression construct comprising a nucleic acid encoding an oncogene, wherein a Lox-STOP-Lox cassette is placed upstream of the transcription or translation initiation site to prevent transcription of the oncogene or translation of the oncogene's transcript; and
   (c) a cancer-prone genetic predisposition,
wherein upon recombination between said two LoxP sites in
the presence of the Cre recombinase, transcription can proceed through said
nucleic acid encoding said oncogene to produce the translated oncoprotein wherein
induced expression of said oncogene causes cell proliferation.

32. The cell according to claim 30 or 31, wherein said
mammary-specific transcriptional element is a mammary-specific promoter or
enhancer.

33. The cell according to claim 32, wherein said mammary-
specific promoter or enhancer is derived from a gene selected from the group
consisting of MMTV LTR, Whey acidic protein, and \( \beta \)-lactoglobulin promoters.

34. A mammalian cell, wherein said cell comprises a genome
comprising:

(a) a nucleic acid encoding an oncogene operably linked
to a nucleic acid encoding an estrogen receptor polypeptide; and

(b) a cancer-prone genetic predisposition,

wherein administration of estrogen or a nonhormone
estrogen analogue to said cell allows the correct folding of the oncogene
polypeptide into a functional protein; and wherein induced expression of said
oncogene causes cell proliferation; and wherein said proliferation is inhibited when
expression of said oncogene is reduced.

35. A mammalian cell, wherein said cell comprises a genome
comprising:

(a) a nucleic acid encoding an oncogene operably linked
to a nucleic acid encoding a progesterone receptor polypeptide; and

(b) a cancer-prone genetic predisposition,

wherein administration of progesterone or a nonhormone
progesterone analogue to said cell allows the correct folding of the oncogene
polypeptide into a functional protein; and wherein induced expression of said
oncogene causes cell proliferation; and wherein said proliferation is inhibited when
expression of said oncogene is reduced.

36. The cell according to any one of claims 28 to 35, wherein
said oncogene is selected from the group consisting of Her2, an activating variant

37. The non-human mammal according to claim 36, wherein said non-Her2 ErbB gene is selected from the group consisting of ErbB1, ErbB3 and ErbB4.

38. The non-human mammal according to claim 36, wherein said viral protein is SV40 T or PyMT.

39. The cell according to claim 28 to 36, wherein said cancer-prone genetic predisposition is a cancer-prone genetic mutation.

40. The cell according to claim 39, wherein the oncogene or the cancer-prone genetic mutation or both the oncogene and the cancer-prone genetic mutation is inducible.

41. The cell according to claim 39, wherein said cancer-prone genetic mutation is selected from the group consisting of (1) a disabling mutation in a tumor suppressor gene, (2) an activating mutation in an endogenous proto-oncogene, (3) a disabling mutation in a DNA repair gene and (4) a disabling or activating mutation in a breast cancer related gene.

42. The cell according to claim 41, wherein said disabling mutation is accomplished by post-transcriptional silencing.

43. The cell according to claim 42, wherein said post-transcriptional silencing is accomplished by RNA interference, antisense or ribozyme.

44. The cell according to any one of claims 41 to 43, wherein said tumor suppressor gene is selected from the group consisting of INK4a, P53, DCC, PTEN, Rb, DPC4, KLF6, GSTP1, ELAC2/HPC2, NKK3.1, LATS, Apaf1, Caspase 8, CHK2, BRCA1, BRCA2, Neurofibromatosis Type 1, Neurofibromatosis Type 2, Adenomatous Polyposis Coli (APC), the Wilms tumor-suppressor gene, Patches and FHIT.
45. The cell according to any one of claims 41 to 44, wherein the proto-oncogene is selected from the group consisting of CTNNB1, myc, and ras.

46. The cell according to any one of claims 41 to 45, wherein the DNA repair gene is selected from the group consisting of MSH2, MSH3, MSH6, PMS2, Ku70, Ku80, DNA/PK, ATR, ATM, XRCC4 and MLH1.

47. The cell according to any one of claims 41 to 46, wherein the breast cancer related gene is selected from the group consisting of Bcl2, cycline B1, myc, ras, estrogen receptor gene and Her2.

48. The cell according to any one of claims 28 to 47, wherein said cell is an ES cell.

49. The cell according to any one of claims 28 to 48, wherein said cell is a mouse cell.

50. A cell derived from the mammal according to any one of claims 1 to 27.

51. The cell according to claim 50, wherein the cell is an ES cell, tumor cell, mammary cell, or tissue-specific stem cell.

52. A mammary tumor explant derived from the mammal according to any one of claims 1 to 27.

53. A method of making the transgenic non-human mammal of claim 23 comprising the step of mating the chimeric non-human mammal of claim 24 with another non-human mammal from the same species.

54. A method of making the non-human mammal of claim 1 comprising the step of mating a first and second non-human mammal, wherein the first mammal comprises a germ cell having a genome comprising a breast cancer related oncogene and the second mammal comprises a germ cell having a genome comprising a cancer-prone genetic predisposition.

55. A method of making the non-human mammal of claim 5 comprising the step of mating a first and a second non-human mammal, wherein the first mammal comprises a germ cell having a genome comprising a nucleic acid encoding reverse tetracycline transactivator under the control of a mammary-specific transcriptional element and wherein the second mammal comprises a germ
cell having a genome comprising an oncogene operably linked to a transcriptional element regulated by said reverse tetracycline transactivator and tetracycline or a tetracycline analogue; and wherein the germ cell of said first mammal or of said second mammal or both further comprises a cancer-prone genetic predisposition.

56. A method of making the non-human mammal of claim 6 comprising the step of mating a first and a second non-human mammal, wherein the first mammal comprises a germ cell having a genome comprising a first expression construct comprising a nucleic acid encoding a Cre recombinase operably linked to a mammary-specific transcriptional element and wherein the second mammal comprises a germ cell having a genome comprising a second expression construct comprising a nucleic acid encoding an oncogene, wherein a Lox-STOP-Lox cassette is placed upstream of the transcription or translation initiation site to prevent transcription of the oncogene or translation of the oncogene's transcript; and wherein the germ cell of said first mammal or of said second mammal or both further comprises a cancer-prone genetic predisposition.

57. The method according to claim 55 or 56, wherein said mammary-specific transcriptional element is a mammary-specific promoter or enhancer.

58. The method according to claim 57, wherein said mammary-specific promoter or enhancer is derived from a gene selected from the group consisting of MMTV LTR, Whey acidic protein, and β-lactoglobulin promoters.

59. A method of making the non-human mammal of claim 9 comprising the step of mating a first and a second non-human mammal, wherein the first mammal comprises a germ cell having a genome comprising an expression construct comprising a nucleic acid encoding an oncogene operably linked to an estrogen receptor polypeptide and wherein the second mammal comprises a germ cell having a genome comprising a cancer-prone genetic predisposition.

60. A method of making the non-human mammal of claim 10 comprising the step of mating a first and a second non-human mammal, wherein the first mammal comprises a germ cell having a genome comprising an expression construct comprising a nucleic acid encoding an oncogene operably linked to a
progesterone receptor polypeptide and wherein the second mammal comprises a
germ cell having a genome comprising a cancer-prone genetic predisposition.

61. A method of making the non-human mammal of claim 1
comprising the steps of:

(a) introducing a construct comprising a breast cancer
related oncogene into a zygote comprising a cancer-prone genetic predisposition;
and

(b) developing the zygote into the non-human mammal.

62. A method of making the non-human mammal of claim 1,
comprising the steps of:

(a) introducing a construct comprising a breast cancer
related oncogene into an ES cell comprising a cancer-prone genetic predisposition;

(b) injecting said ES cell into a blastocyst or a tetraploid
blastocyst; and

(c) generating the non-human mammal.

63. The method according to claims 61 or 62, wherein the non-
human mammal is a chimeric mammal.

64. The method according to any one of claims 53 to 63,
wherein said oncogene is selected from the group consisting of Her2, an activating
variant of Her2, Bcl2, cyclin D1, myc, H-ras, K-ras, estrogen receptor gene,
progesterone receptor gene, a non-Her2 ErbB gene, a gene in the MAPK signal
transduction pathway, a gene in the PI3K-AKT signal transduction pathway,
TGFα, PI3K, ras-GAP, Shc, Nck, src, Yes, Fyn, β-catenin, and a viral protein.

65. The non-human mammal according to claim 64, wherein
said non-Her2 ErbB gene is selected from the group consisting of ErbB1, ErbB3
and ErbB4.

66. The non-human mammal according to claim 64, wherein
said viral protein is SV40 T or PyMT.

67. The method according to any one of claims 53 to 66,
wherein a reporter gene is operably linked to said oncogene.
68. The method according to any one of claims 53 to 67, wherein said cancer-prone genetic predisposition is a cancer-prone genetic mutation.

69. The method according to claim 68, wherein said cancer-prone genetic mutation is selected from the group consisting of (1) a disabling mutation in a tumor suppressor gene, (2) an activating mutation in an endogenous proto-oncogene, (3) a disabling mutation in a DNA repair gene and (4) a disabling or activating mutation in a breast cancer related gene.

70. The method according to claim 69, wherein said disabling mutation is accomplished by post-transcriptional silencing.

71. The method according to claim 70, wherein said post-transcriptional silencing is accomplished by RNA interference, antisense or ribozyme.

72. The method according to any one of claims 69 to 71, wherein said tumor suppressor gene is selected from the group consisting of INK4a, P53, DCC, PTEN, Rb, DPC4, KLF6, GSTP1, ELAC2/HPC2, NXX3.1, LATS, Apaf1, Caspase 8, CHK2, BRCA1, BRCA2, Neurofibromatosis Type 1, Neurofibromatosis Type 2, Adenomatous Polyposis Coli (APC), the Wilms tumor-suppressor gene, Patches and FHIT.

73. The non-human mammal according to any one of claims 69 to 72, wherein the proto-oncogene is selected from the group consisting of CTNNB1, myc, and ras.

74. The non-human mammal according to any one of claims 69 to 73, wherein the DNA repair gene is selected from the group consisting of MSH2, MSH3, MSH6, PMS2, Ku70, Ku80, DNA/PK, ATR, ATM, XRCC4 and MLH1.

75. The non-human mammal according to any one of claims 69 to 74, wherein the breast cancer related gene is selected from the group consisting of Bcl2, cycline B1, myc, ras, estrogen receptor gene and Her2.

76. The method according to any one of claims 53 to 75, wherein said mammal is a mouse.
77. A method of making a non-human mammal of claim 1 comprising the step of generating a non-human mammal from the ES cell of claim 48.

78. A method for determining whether an oncogene contributes to breast cancer maintenance, comprising the steps of:

   (a) providing a non-human mammal having breast cancer, wherein said mammal comprises a genome comprising:

       (i) said oncogene operably linked to an inducible promoter; and

       (ii) a cancer-prone genetic predisposition wherein said mammal comprises a mammary tumor formed in said mammal during expression of said oncogene; and

   (b) determining whether or not said mammary tumor regresses when expression of said oncogene is reduced, wherein mammary tumor regression is indicative of said oncogene contributing to breast cancer maintenance.

79. A method of identifying a breast cancer related gene, comprising the steps of:

   (a) establishing a first and a second molecular profile of a mammary tumor cell of the non-human mammal of claim 1 or the cell of claim 28 at two different stages of breast cancer; and

   (b) comparing the first and second molecular profiles, wherein an alteration in expression or activity pattern of a candidate gene is indicative of said gene being a breast cancer related gene.

80. A method of identifying a biomarker to diagnose or monitor breast cancer progression, comprising the steps of:

   (a) establishing a first molecular profile of a mammary cell of the non-human mammal of claim 1 or the cell of claim 28 wherein expression of the breast cancer related oncogene is not induced and wherein the cell becomes cancerous;
(b) establishing a second molecular profile of said cell wherein expression of said oncogene is induced and wherein the cell becomes cancerous; and

(c) comparing the first and second molecular profiles, wherein an alteration in expression or activity pattern of a candidate gene is indicative of said gene being a biomarker for breast cancer.

81. A method of identifying a therapeutic agent to treat breast cancer, comprising the steps of:

(a) administering a candidate compound to the non-human mammal of claim 1 that has developed breast cancer; and

(b) observing the effect of said compound on cancer development,

wherein a decrease in tumor size, metastasis, angiogenesis or growth rate, or apoptosis of the cancer is indicative of said compound being a therapeutic agent to treat breast cancer.

82. A method of identifying a therapeutic agent to prevent breast cancer, comprising the steps of:

(a) administering a candidate compound to the non-human mammal of claim 1 that has not yet developed breast cancer;

(b) inducing expression of the breast cancer related oncogene to cause cancer; and

(c) observing the effect of said compound on cancer development,

wherein absence of cancer formation is indicative of said compound being a therapeutic agent to prevent breast cancer.

83. A method of identifying a therapeutic agent to treat breast cancer, comprising the steps of:

(a) contacting the cell of claim 28 with a candidate compound wherein said cell is a mammary tumor cell; and

(b) observing the effect of said compound on cell proliferation,
wherein inhibition of cell proliferation is indicative of said compound being a therapeutic agent to treat breast cancer.

84. A method of identifying a therapeutic agent to treat breast cancer, comprising the steps of:
   
   (a) administering a candidate compound to the non-human mammal of claim 1 that has developed breast cancer or to cultured breast cancer cells derived from said mammal;
   
   (b) inducing expression of the breast cancer related oncogene to cause cancer; and
   
   (c) observing the effect of said compound on expression or activity level of a biomarker for breast cancer in said mammal or said cells,

wherein an alteration of biomarker expression or activity is indicative of said compound being a therapeutic agent to treat breast cancer.

85. A method of identifying a therapeutic agent to prevent breast cancer, comprising the steps of:

   (a) administering a candidate compound to the non-human mammal of claim 1 that has not yet developed breast cancer or to cultured breast cancer cells derived from said mammal;

   (b) inducing expression of the breast cancer related oncogene to cause cancer; and

   (c) observing the effect of said compound on expression or activity level of a biomarker for breast cancer in said mammal or said cells,

wherein absence of alteration of biomarker expression or activity is indicative of said compound being a therapeutic agent to treat breast cancer.

86. A method of identifying a therapeutic agent to treat breast cancer, comprising the steps of:

   (a) contacting the cell of claim 28 with a candidate compound wherein the cell is a mammary tumor cell; and

   (b) observing the effect of said compound on expression or activity level of a biomarker for breast cancer in said cells,
wherein an alteration of biomarker expression or activity is indicative of said compound being a therapeutic agent to treat breast cancer.

87. A method of identifying a therapeutic agent to prevent breast cancer, comprising the steps of:
   (a) establishing a first molecular profile of a non-cancerous mammary cell of the non-human mammal of claim 1, or a mammary cell derived from said mammal, by identifying a plurality of biomarkers whose patterns of expression or biological activity correspond to the non-cancerous stage of said mammary cell, and wherein expression of the breast cancer related oncogene is not induced;
   (b) contacting said mammary cell with a candidate compound;
   (c) establishing a second molecular profile of said contacted mammary cell, wherein said second pattern of expression or biological activity of said biomarkers correspond to said mammary cell, wherein expression of said oncogene is induced to cause cancer; and
   (d) comparing the first and second profiles, wherein substantial similarity of the first and second profiles is indicative of said compound being a therapeutic agent to prevent breast cancer.

88. A method of identifying a gene involved in minimal residual breast cancer, comprising the steps of:
   (a) establishing a first molecular profile for a non-cancerous mammary cell of the non-human mammal of claim 1; and
   (b) establishing a second molecular profile for a mammary cell of the non-human mammal of claim 1 having minimal residual breast cancer;
   (c) establishing a third molecular profile for a cancerous mammary cell of the non-human mammal of claim 1 wherein cancer is induced by expression of the breast cancer related oncogene; and
   (d) comparing the first, second and third profiles, wherein an alteration in expression patterns of said gene in the first and second profiles while substantial similarity of expression patterns of
said gene in the second and third profiles is indicative of said gene being involved in minimal residual breast cancer.

89. A method of identifying a therapeutic agent to treat or prevent minimal residual breast cancer, comprising the steps of:

(a) administering a candidate compound to the non-human mammal of claim 1, wherein the mammal has minimal residual breast cancer; and

(b) observing the effect of said compound on expression or activity level of a gene involved in minimal residual breast cancer,

wherein an alteration in expression or activity level of said gene is indicative of said compound being a therapeutic agent to treat or prevent minimal residual breast cancer.

90. The method according to any one of claims 78 to 89, wherein said cancer-prone genetic predisposition is a cancer-prone genetic mutation.

91. The method according to claim 90, wherein the cancer-prone genetic mutation is inducible.

92. The method according to claim 90, wherein said cancer-prone genetic mutation is selected from the group consisting of (1) a disabling mutation in a tumor suppressor gene, (2) an activating mutation in an endogenous proto-oncogene, (3) a disabling mutation in a DNA repair gene and (4) a disabling or activating mutation in a breast cancer related gene.

93. The method according to claim 92, wherein said disabling mutation is accomplished by post-transcriptional silencing.

94. The method according to claim 93, wherein said post-transcriptional silencing is accomplished by RNA interference, antisense or ribozyme.

95. The method according to any one of claims 92 to 94, wherein said tumor suppressor gene is selected from the group consisting of INK4a, P53, DCC, PTEN, Rb, DPC4, KLF6, GSTP1, ELAC2/HPC2, NIX3.1, LATS, Apaf1, Caspase 8, CHK2, BRCA1, BRCA2, Neurofibromatosis Type 1,
Neurofibromatosis Type 2, Adenomatous Polyposis Coli (APC), the Wilms tumor-suppressor gene, Patches and FHIT.

96. The method according to any one of claims 92 to 95, wherein the proto-oncogene is selected from the group consisting of CTNNB1, myc, ras and her2.

97. The method according to any one of claims 92 to 96, wherein the DNA repair gene is selected from the group consisting of MSH2, MSH3, MSH6, PMS2, Ku70, Ku80, DNA/PK, ATR, ATM, XRCC4 and MLH1.

98. The method according to any one of claims 92 to 97, wherein the breast cancer related gene is selected from the group consisting of Bcl2, cycline B1, myc, ras, estrogen receptor gene and Her2.

99. The method according to any one of claims 79 to 87 wherein said oncogene is selected from the group consisting of Her2, an activating variant of Her2, Bcl2, cyclin D1, myc, H-ras, K-ras, estrogen receptor gene, progesterone receptor gene, a non-Her2 ErbB gene, a gene in the MAPK signal transduction pathway, a gene in the PI3K-AKT signal transduction pathway, TGFβ, PI3K, ras-GAP, Shc, Nck, src, Yes, Fyn, β-catenin, and a viral protein.

100. The method according to any one of claims 78 to 89, wherein a reporter gene is operably linked to said oncogene.

101. The method according to any one of claims 78, 79, 80, 81, 82, 84, 85, 87, 88 or 89, wherein said mammal is a mouse.

102. The method according to claim 79, 80, 87 or 88, wherein said profiles are established by a technique selected from the group consisting of suppression subtraction, differential display, proteomic analysis, serial analysis of gene expression and comparative genomic hybridization.
Figure 1
Figure 2