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Pan et al.

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(54) **METHODS AND COMPOSITIONS RELATED TO GLUCOCORTICOID RECEPTOR ANTAGONISTS AND BREAST CANCER**

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 220 days.

This patent is subject to a terminal disclaimer.

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Related U.S. Application Data

(63) Continuation of application No. 15/448,827, filed on Mar. 3, 2017, now Pat. No. 10,441,596, which is a continuation of application No. 14/296,127, filed on Jun. 4, 2014, now Pat. No. 9,623,032, which is a continuation of application No. 14/172,051, filed on Feb. 4, 2014, now Pat. No. 9,149,485, which is a continuation of application No. 13/071,363, filed on Mar. 24, 2011, now Pat. No. 8,710,035.

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(51) **Int. Cl.**

A61K 31/575	(2006.01)
A61K 45/06	(2006.01)
A61K 33/243	(2019.01)
A61K 31/282	(2006.01)
A61K 31/337	(2006.01)
A61K 31/357	(2006.01)
A61K 31/4745	(2006.01)
A61K 31/7068	(2006.01)
A61K 39/395	(2006.01)
A61N 5/00	(2006.01)
A61J 1/00	(2006.01)
A61K 31/567	(2006.01)
A61K 31/58	(2006.01)

(52) **U.S. Cl.**

CPC ***A61K 31/575*** (2013.01); ***A61J 1/00*** (2013.01); ***A61K 31/282*** (2013.01); ***A61K 31/337*** (2013.01); ***A61K 31/357*** (2013.01); ***A61K 31/4745*** (2013.01); ***A61K 31/567*** (2013.01); ***A61K 31/58*** (2013.01); ***A61K 31/7068*** (2013.01); ***A61K 33/243*** (2019.01); ***A61K 39/3955*** (2013.01); ***A61K 45/06*** (2013.01); ***A61N 5/00*** (2013.01)

(58) **Field of Classification Search**

CPC A61K 31/575; A61K 45/06; A61K 33/243; A61K 31/282; A61K 31/337; A61K 31/357; A61K 31/4745; A61K 31/7068; A61K 39/395; A61K 31/567; A61K 31/56; A61N 5/00; A61J 1/00

See application file for complete search history.

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(Continued)

Primary Examiner — Shirley V Gembeh

(74) *Attorney, Agent, or Firm* — Kilpatrick Townsend & Stockton LLP

(57) **ABSTRACT**

Embodiments of the invention are directed to methods of determining the prognosis of a breast cancer patient by evaluating the activity of the glucocorticoid receptor in tumor cells. Other embodiment include methods of treating breast cancer cells, particularly, chemo-resistant cells, with a glucocorticoid receptor antagonist and an anticancer agent or compound.

20 Claims, 12 Drawing Sheets

Specification includes a Sequence Listing.

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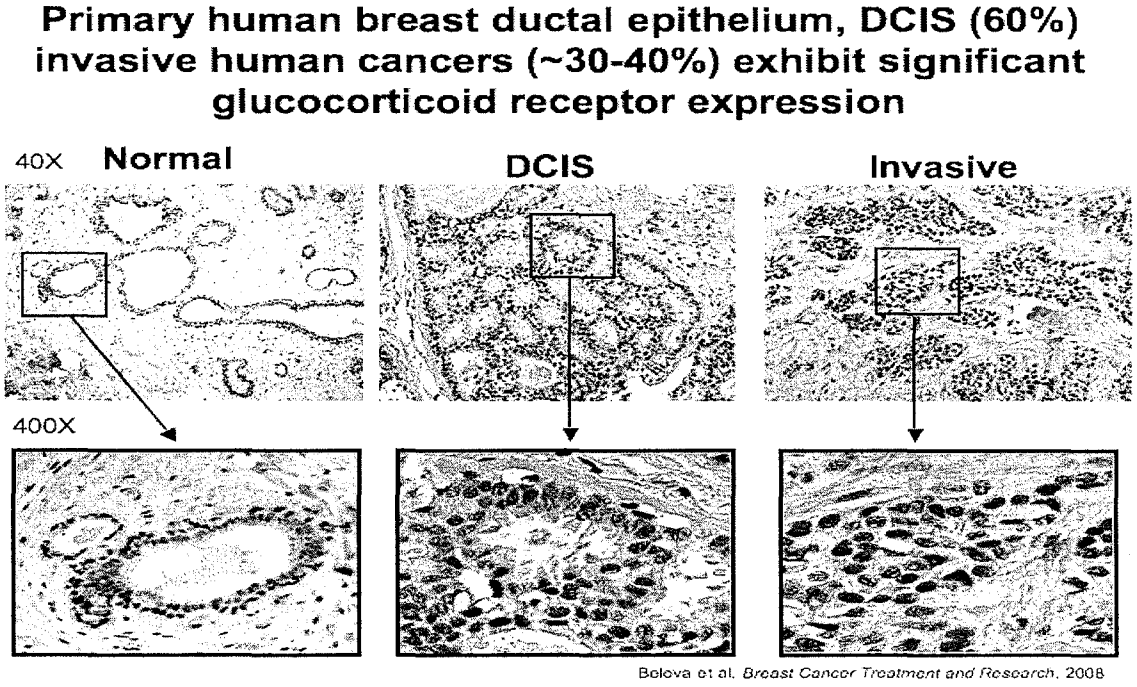

Primary human breast ductal epithelium, DCIS (60%) invasive human cancers (~30-40%) exhibit significant glucocorticoid receptor expression
40X
Normal
DCIS
Invasive
400X
Beleva et al. Breast Cancer Treatment and Research. 2008

FIG. 1

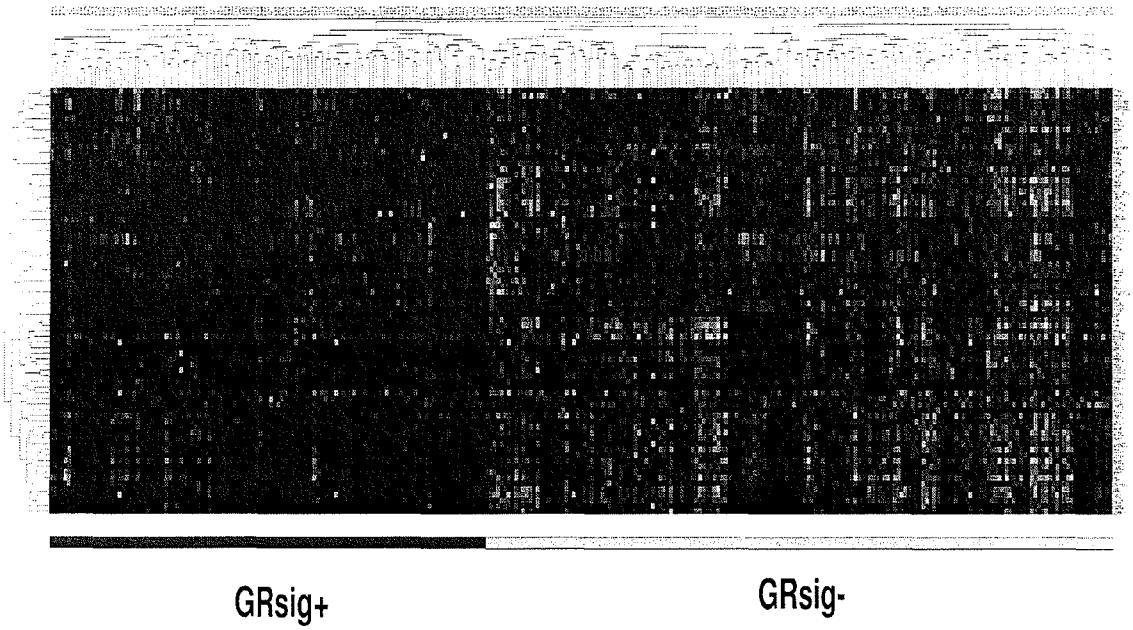

GRsig+
GRsig-

FIG. 2

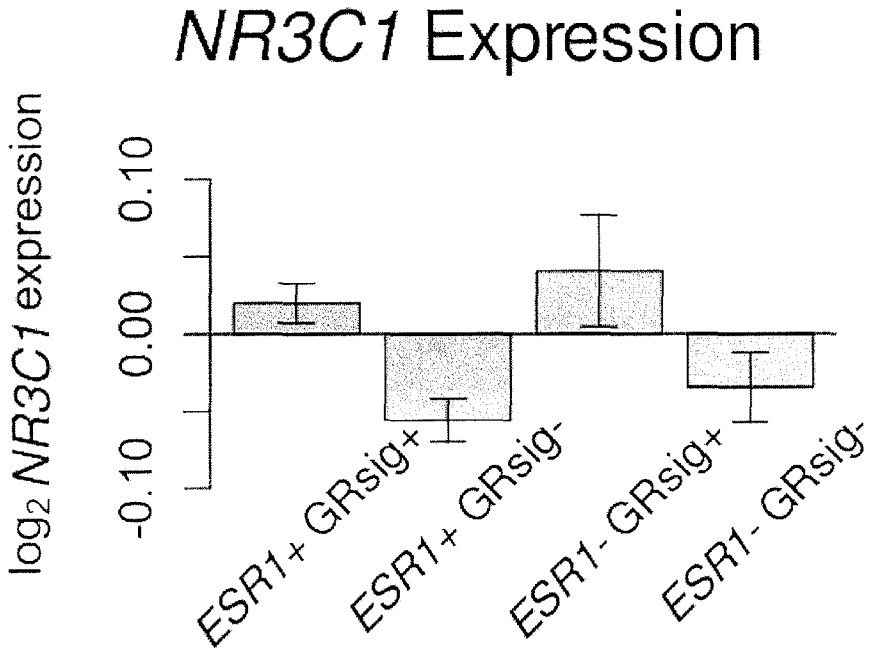

NR3C1 Expression
log2 NR3C1 expression
0.10
0.00
-0.10
ESR1+ GRsig+
ESR1+ GRsig-
ESR1- GRsig+
ESR1- GRsig-

FIG. 3

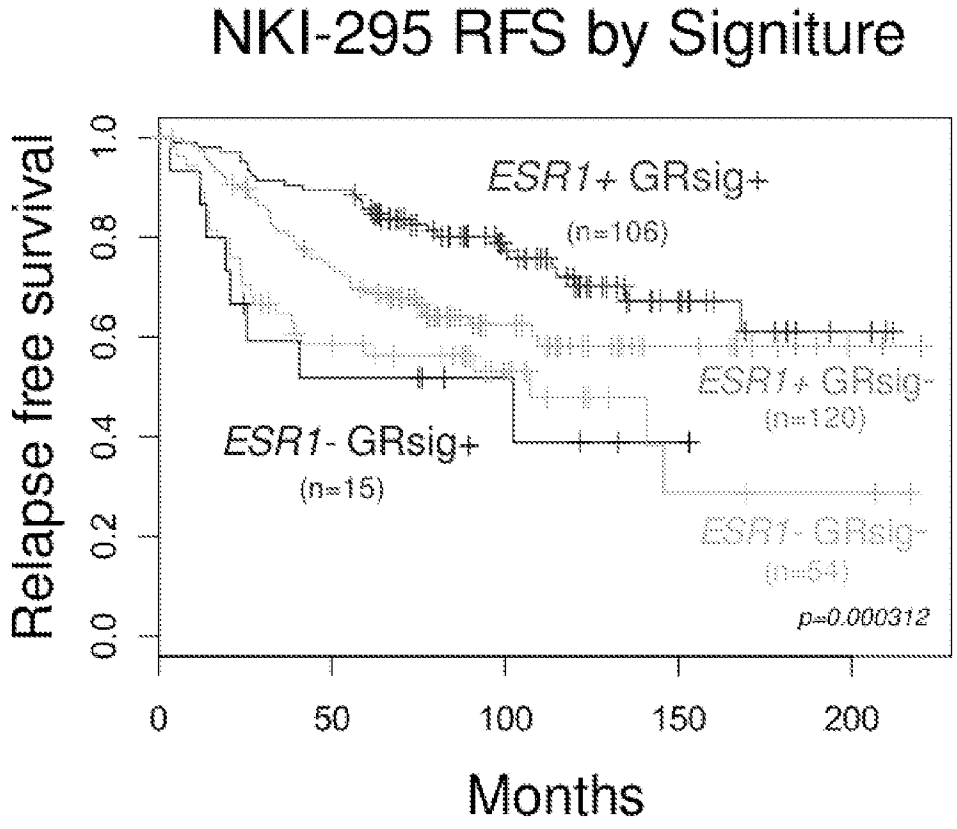

NKI-295 RFS by Signiture
Relapse free survival
0.0
0.2
0.4
0.6
0.8
1.0
ESR1+ GRsig+
(n=106)
ESR1+ GRsig-
(n=120)
ESR1- GRsig+
(n=15)
ESR1- GRsig-
(n=64)
p=0.000312
0
50
100
150
200
Months

FIG. 4

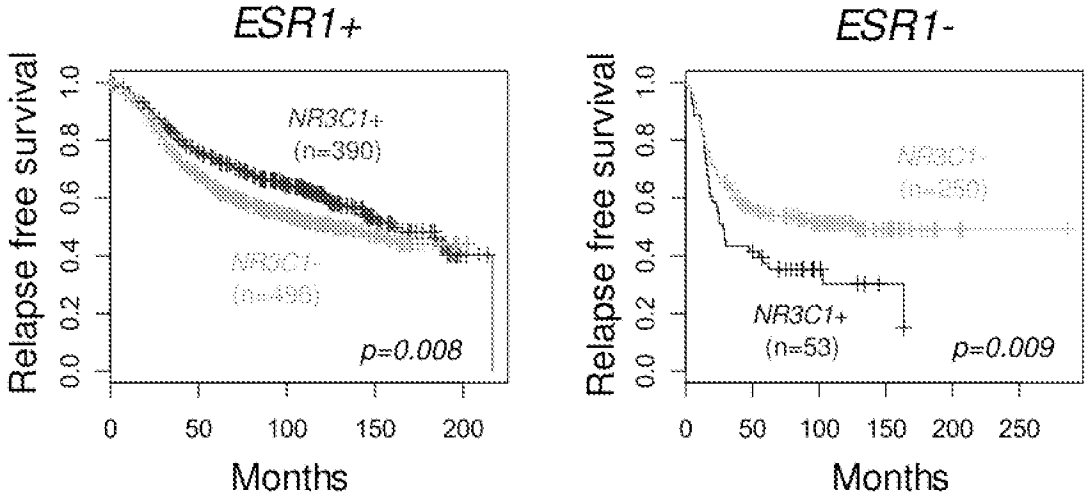

ESR1+
Relapse free survival
0.0
0.2
0.4
0.6
0.8
1.0
NR3C1+
(n=390)
NR3C1-
(n=490)
p=0.008
0
50
100
150
200
Months
ESR1-
Relapse free survival
0.0
0.2
0.4
0.6
0.8
1.0
NR3C1-
(n=250)
NR3C1+
(n=53)
p=0.009
0
50
100
150
200
250
Months

FIG. 5

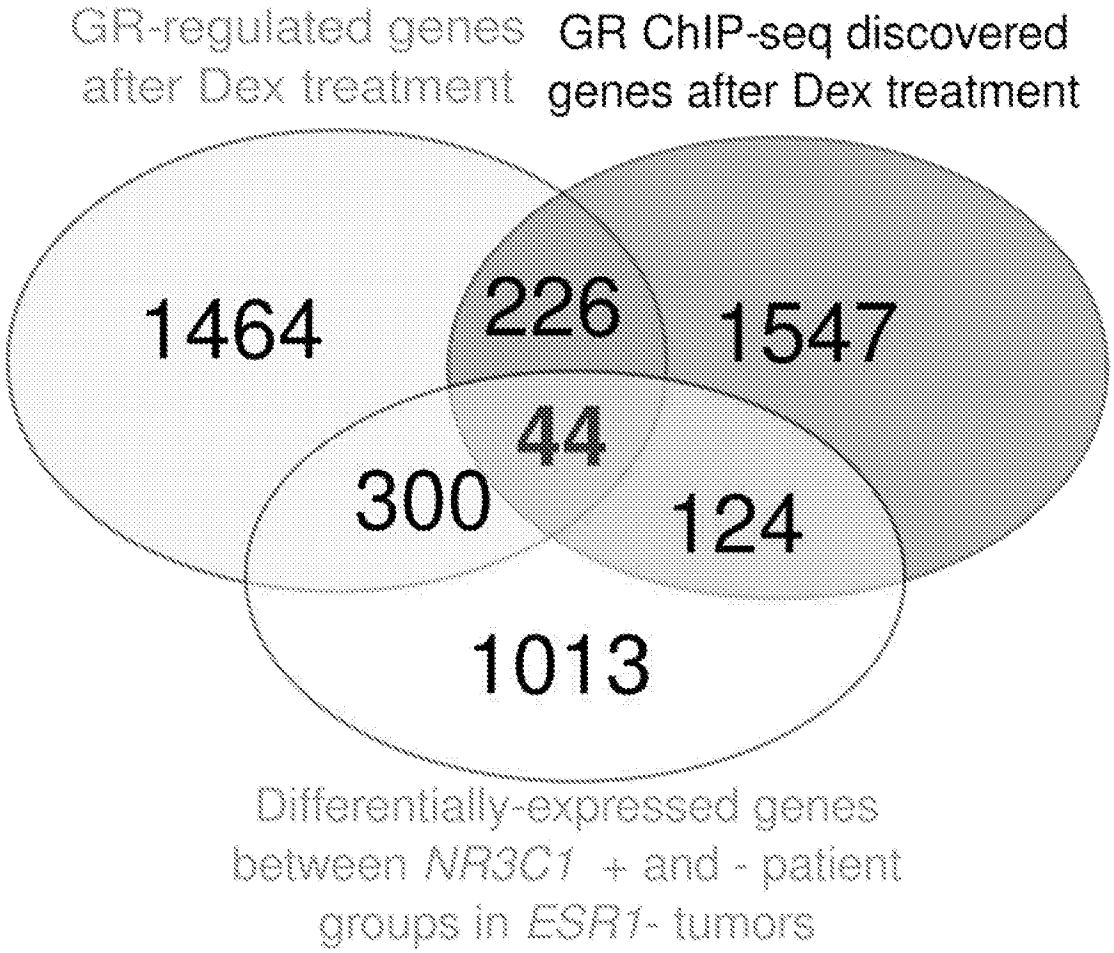
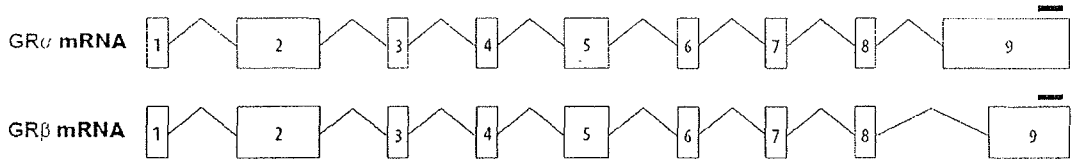

GR-regulated genes after Dex treatment
GR ChIP-seq discovered genes after Dex treatment
1464
226
1547
44
300
124
1013
Differentially-expressed genes between NR3C1 + and - patient groups in ESR1- tumors

FIG. 6

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Length=6784

18665 = GR beta

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18665  61     TTCGCGTGTCCGCGCTCTCTTCCCTCCGCCGCCGCCTCCTCCATTTTGCGAGCTCGTGTC  120

Query  121    TGTGACGGGAGCCCGAGTCACCGCCTGCCCGTCGGGGACGGATTCTGTGGGTGGAAGGAG  180
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Query  301    CTTTCTTAAATAGGGGCTCTCCCCCCACCCATGGAGAAAGGGGCGGCTGTTTACTTCCtt  360
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18665  361    TTTTTAGAAAAAAAAAAATATATTTCCCTCCTGCTCCTTCTGCGTTCACAAGCTAAGTTGT  420

Query  421    TTATCTCGGCTGCGGCGGGAACTGCGGACGGTGGCGGGCGAGCGGCTCCTCTGCCAGAGT  480
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18665  481    TGATATTCACTGATGGACTCCAAAGAATCATTAACTCCTGGTAGAGAAGAAAACCCCAGC  540

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Query  601    GCTACTGTGAAGGTTTCTGCGTCTTCACCCTCACTGGCTGTCGCTTCTCAATCAGACTCC  660
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FIG. 7A

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Query  2041  AACAAAACAATAGTTCCTGCAACGTTACCACAACTCACCCCTACCCTGGTGTCACTGTTG  2100
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FIG. 7B

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Query  2161  TGGAGGATCATGACTACGCTCAACATGTTAGGAGGGCGGCAAGTGATTGCAGCAGTGAAA  2220
18665  2161  TGGAGGATCATGACTACGCTCAACATGTTAGGAGGGCGGCAAGTGATTGCAGCAGTGAAA  2220

Query  2221  TGGGCAAAGGCAATACCAGGTTTCAGGAACTTACACCTGGATGACCAAATGACCCTACTG  2280
18665  2221  TGGGCAAAGGCAATACCAGGTTTCAGGAACTTACACCTGGATGACCAAATGACCCTACTG  2280

Query  2281  CAGTACTCCTGGATGTTTCTTATGGCATTTGCTCTGGGGTGGAGATCATATAGACAATCA  2340
18665  2281  CAGTACTCCTGGATGTTTCTTATGGCATTTGCTCTGGGGTGGAGATCATATAGACAATCA  2340

Query  2341  AGTGCAAACCTGCTGTGTTTTGCTCCTGATCTGATTATTAATGAGCAGAGAATGACTCTA  2400
18665  2341  AGTGCAAACCTGCTGTGTTTTGCTCCTGATCTGATTATTAATGAGCAGAGAATGACTCTA  2400

Query  2401  CCCTGCATGTACGACCAATGTAAACACATGCTGTATGTTTCCTCTGAGTTACACAGGCTT  2460
18665  2401  CCCTGCATGTACGACCAATGTAAACACATGCTGTATGTTTCCTCTGAGTTACACAGGCTT  2460

Query  2461  CAGGTATCTTATGAAGAGTATCTCTGTATGAAAACCTTACTGCTTCTCTCTTCAGTTCCT  2520
18665  2461  CAGGTATCTTATGAAGAGTATCTCTGTATGAAAACCTTACTGCTTCTCTCTTCAGTTCCT  2520

Query  2521  AAGGACGGTCTGAAGAGCCAAGAGCTATTTGATGAAATTAGAATGACCTACATCAAAGAG  2580
18665  2521  AAGGACGGTCTGAAGAGCCAAGAGCTATTTGATGAAATTAGAATGACCTACATCAAAGAG  2580

Query  2581  CTAGGAAAAGCCATTGTCAAGAGGGAAGGAAACTCCAGCCAGAACTGGCAGCGGTTTTAT  2640
18665  2581  CTAGGAAAAGCCATTGTCAAGAGGGAAGGAAACTCCAGCCAGAACTGGCAGCGGTTTTAT  2640

Query  2641  CAACTGACAAAACTCTTGGATTCTATGCATGAAGTGGTTGAAAATCTCCTTAACTATTGC  2700
18665  2641  CAACTGACAAAACTCTTGGATTCTATGCATGAA                             2673

Query  2701  TTCCAAACATTTTTGGATAAGACCATGAGTATTGAATTCCCCGAGATGTTAGCTGAAATC  2760

Query  2761  ATCACCAATCAGATACCAAAATATTCAAATGGAAATATCAAAAAACTTCTGTTTCATCAA  2820

Query  2821  AAGTGACTGCCTTAATAAGAATGGTTGCCTTAAAGAAAGTCGAATTAATAGCTTTTATTG  2880

Query  2881  TATAAACTATCAGTTTGTCCTGTAGAGgttttgttgttttatttttttattgttttcatct  2940

Query  2941  gttgttttgttttAAATACGCACTACATGTGGTTTATAGAGGGCCAAGACTTGGCAACAG  3000

Query  3001  AAGCAGTTGAGTCGTCATCACTTTTCAGTGATGGGAGAGTAGATGGTGAAATTTATTAGT  3060

Query  3061  TAATATATCCCAGAAATTAGAAACCTTAATATGTGGACGTAATCTCCACAGTCAAAGAAG  3120

Query  3121  GATGGCACCTAAACCACCAGTGCCCAAAGTCTGTGTGATGAACTTTCTCTTCATACtttt  3180

Query  3181  tttCACAGTTGGCTGGATGAAATTTTCTAGACTTTCTGTTGGTGTATccccccccTGTAT  3240

Query  3241  AGTTAGGATAGCATTTTTGATTTATGCATGGAAACCTGaaaaaaaGTTTACAAGTGTATA  3300

Query  3301  TCAGAAAAGGGAAGTTGTGCCTTTTATAGCTATTACTGTCTGGTTTTAACAATTTCCTTT  3360

Query  3361  ATATTTAGTGAACTACGCTTGCTCATTTTTTCTTACATAATTTTTTATTCAAGTTATTGT  3420

Query  3421  ACAGCTGTTTAAGATGGGCAGCTAGTTCGTAGCTTTCCCAAATAAACTCTAAACATTAAT  3480

Query  3481  CAATCATCTGTGTGAAAATGGGTTGGTGCTTCTAACCTGATGGCACTTAGCTATCAGAAG  3540

Query  3541  ACCACAAAAATTGACTCAAATCTCCAGTATTCTTGTCaaaaaaaaaaaaaaaaaaGCTCA  3600

Query  3601  TATTTTGTATATATCTGCTTCAGTGGAGAATTATATAGGTTGTGCAAATTAACAGTCCTA  3660

Query  3661  ACTGGTATAGAGCACCTAGTCCAGTGACCTGCTGGGTAAACTGTGGATGATGGTTGCAAA  3720

Query  3721  AGACTAATTTAAAAAATAACTACCAAGAGGCCCTGTCTGTACCTAACGCCCTATTTTTGC  3780
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FIG. 7C

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Query  3781  AATGGCTATATGGCAAGAAAGCTGGTAAACTATTTGTCTTTCAGGACCTTTTGAAGTAGT  3840

Query  3841  TTGTATAACTTCTTAAAAGTTGTGATTCCAGATAACCAGCTGTAACACAGCTGAGAGACT  3900

Query  3901  TTTAATCAGACAAAGTAATTCCTCTCACTAAACTTTACCCAAAAACTAAATCTCTAATAT  3960

Query  3961  GGCAAAAATGGCTAGACACCCATTTTCACATTCCCATCTGTCACCAATTGGTTAATCTTT  4020

Query  4021  CCTGATGGTACAGGAAAGCTCAGCTACTGATTTTTGTGATTTAGAACTGTATGTCAGACA  4080

Query  4081  TCCATGTTTGTAAAACTACACATCCCTAATGTGTGCCATAGAGTTTAACACAAGTCCTGT  4140

Query  4141  GAATTTCTTCACTGTTGAAAATTATTTTAAACAAAATAGAAGCTGTAGTAGCCCTTTCTG  4200

Query  4201  TGTGCACCTTACCAACTTTCTGTAAACTCAAAACTTAACATATTTACTAAGCCACAAGAA  4260

Query  4261  ATTTGATTTCTATTCAAGGTGGCCAAATTATTTGTGTAATAGAAAACTGAAAATCTAATA  4320

Query  4321  TTAAAAATATGGAACTTCTAatatatttttatatttagttatagtttcagatatatatca  4380

Query  4381  tatTGGTATTCACTAATCTGGGAAGGGAAGGGCTACTGCAGCTTTACATGCAATTTATTA  4440

Query  4441  AAATGATTGTAAAATAGCTTGTATAGTGTAAAATAAGAATGATTTTTAGATGAGATTGTT  4500

Query  4501  TTATCATGACATGTTATATATTTTTTGTAGGGGTCAAAGAAATGCTGATGGATAACCTAT  4560

Query  4561  ATGATTTATAGTTTGTACATGCATTCATACAGGCAGCGATGGTCTCAGAAACCAAACAGT  4620

Query  4621  TTGCTCTAGGGGAAGAGGGAGATGGAGACTGGTCCTGTGTGCAGTGAAGGTTGCTGAGGC  4680

Query  4681  TCTGACCCAGTGAGATTACAGAGGAAGTTATCCTCTGCCTCCCATTCTGACCACCCTTCT  4740

Query  4741  CATTCCAACAGTGAGTCTGTCAGCGCAGGTTTAGTTTACTCAATCTCCCCTTGCACTAAA  4800

Query  4801  GTATGTAAAGTATGTAAACAGGAGACAGGAAGGTGGTGCTTACATCCTTAAAGGCACCAT  4860

Query  4861  CTAATAGCGGGTTACTTTCACATACAGCCCTCCCCCAGCAGTTGAATGACAACAGAAGCT  4920

Query  4921  TCAGAAGTTTGGCAATAGTTTGCATAGAGGTACCAGCAATATGTAAATAGTGCAGAATCT  4980

Query  4981  CATAGGTTGCCAATAATACACTAATTCCTTTCTATCCTACAACAAGAGTTTATTTCCAAA  5040

Query  5041  TAAAATGAGGACAtgtttttgttttctttgaatgctttttgaatgttatttgttatttttc  5100

Query  5101  agtattttggagaaattatttAATaaaaaaaCAATCATTTGCTTTTTGAATGCTCTCTAA  5160

Query  5161  AAGGGAATGTAATATTTTAAGATGGTGTGTAACCCGGCTGGATAAATTTTTGGTGCCTAA  5220

Query  5221  GAAAACTGCTTGAATATTCTTATCAATGACAGTGTTAAGTTTCAAAAAGAGCTTCTAAAA  5280

Query  5281  CGTAGATTATCATTCCTTTATAGAATGTTATGTGGTTAAAACCAGAAAGCACATCTCACA  5340
18665  2674                          AATGTTATGTGGTTAAAACCAGAAAGCACATCTCACA  2710

Query  5341  CATTAATCTGATTTTCATCCCAACAATCTTGGCGCTCAAAAAATAGAACTCAATGAGAAA  5400
18665  2711  CATTAATCTGATTTTCATCCCAACAATCTTGGCGCTCAAAAAATAGAACTCAATGAGAAA  2770

Query  5401  AAGAAGATTATGTGCACTTCGTTGTCAATAATAAGTCAACTGATGCTCATCGACAACTAT  5460
18665  2771  AAGAAGATTATGTGCACTTCGTTGTCAATAATAAGTCAACTGATGCTCATCGACAACTAT  2830

Query  5461  AGGAGGCTTTTTCATTAAATGGGAAAAGAAGCTGTGCCCTTTTAGGATACGTGGGGGAAAA  5520
18665  2831  AGGAGGCTTTTTCATTAAATGGGAAAAGAAGCTGTGCCCTTTTAGGATACGTGGGGGAAAA  2890

Query  5521  GAAAGTCATCTTAATTATGTTTAATTGTGGATTTAAGTGCTATATGGTGGTGCTGTTTGA  5580
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FIG. 7D

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18665  2891  GAAAGTCATCTTAATTATGTTTAATTGTGGATTTAAGTGCTATATGGTGGTGCTGTTTGA  2950

Query  5581  AAGCAGATTTATTTCCTATGTATGTGTTATCTGGCCATCCCAACCCAAACTGTTGAAGTT  5640
18665  2951  AAGCAGATTTATTTCCTATGTATGTGTTATCTGGCCATCCCAACCCAAACTGTTGAAGTT  3010

Query  5641  TGTAGTAACTTCAGTGAGAGTTGGTTACTCACAACAAATCCTGAAAAGTATTTTTAGTGT  5700
18665  3011  TGTAGTAACTTCAGTGAGAGTTGGTTACTCACAACAAATCCTGAAAAGTATTTTTAGTGT  3070

Query  5701  TTGTAGGTATTCTGTGGGATACTATACAAGCAGAACTGAGGCACTTAGGACATAACACTT  5760
18665  3071  TTGTAGGTATTCTGTGGGATACTATACAAGCAGAACTGAGGCACTTAGGACATAACACTT  3130

Query  5761  TTGGGGTATATATATCCAAATGCCTAAAACTATGGGAGGAAACCTTGGCCACCCCAAAAG  5820
18665  3131  TTGGGGTATATATATCCAAATGCCTAAAACTATGGGAGGAAACCTTGGCCACCCCAAAAG  3190

Query  5821  GAAAACTAACATGATTTGTGTCTATGAAGTGCTGGATAATTAGCATGGGATGAGCTCTGG  5880
18665  3191  GAAAACTAACATGATTTGTGTCTATGAAGTGCTGGATAATTAGCATGGGATGAGCTCTGG  3250

Query  5881  GCATGCCATGAAGGAAAGCCACGCTCCCTTCAGAATTCAGAGGCAGGGAGCAATTCCAGT  5940
18665  3251  GCATGCCATGAAGGAAAGCCACGCTCCCTTCAGAATTCAGAGGCAGGGAGCAATTCCAGT  3310

Query  5941  TTCACCTAAGTCTCATAATTTTAGTTCCCTTTTAAAAACCCTGAAAACTACATCACCATG  6000
18665  3311  TTCACCTAAGTCTCATAATTTTAGTTCCCTTTTAAAAACCCTGAAAACTACATCACCATG  3370

Query  6001  GAATGAAAAATATTGTTATACAATACATTGATCTGTCAAACTTCCAGAACCATGGTAGCC  6060
18665  3371  GAATGAAAAATATTGTTATACAATACATTGATCTGTCAAACTTCCAGAACCATGGTAGCC  3430

Query  6061  TTCAGTGAGATTTCCATCTTGGCTGGTCACTCCCTGACTGTAGCTGTAGGTGAAtgtgtt  6120
18665  3431  TTCAGTGAGATTTCCATCTTGGCTGGTCACTCCCTGACTGTAGCTGTAGGTGAATGTGTT  3490

Query  6121  tttgtgtgtgtgtgtCTGGTTTTAGTGTCAGAAGGGAAATAAAAGTGTAAGGAGGACACT  6180
18665  3491  TTTGTGTGTGTGTGTCTGGTTTTAGTGTCAGAAGGGAAATAAAAGTGTAAGGAGGACACT  3550

Query  6181  TTAAACCCTTTGGGTGGAGTTTCGTAATTTCCCAGACTATTTTCAAGCAACCTGGTCCAC  6240
18665  3551  TTAAACCCTTTGGGTGGAGTTTCGTAATTTCCCAGACTATTTTCAAGCAACCTGGTCCAC  3610

Query  6241  CCAGGATTAGTGACCAGGTTTTCAGGAAAGGATTTGCTTCTCTCTAGAAAATGTCTGAAA  6300
18665  3611  CCAGGATTAGTGACCAGGTTTTCAGGAAAGGATTTGCTTCTCTCTAGAAAATGTCTGAAA  3670

Query  6301  GGATTTTATTTTCTGATGAAAGGCTGTATGAAAATACCCTCCTCAAATAACTTGCTTAAC  6360
18665  3671  GGATTTTATTTTCTGATGAAAGGCTGTATGAAAATACCCTCCTCAAATAACTTGCTTAAC  3730

Query  6361  TACATATAGATTCAAGTGTGTCAATATTCTATTTTGTATATTAAATGCTATATAATGGGG  6420
18665  3731  TACATATAGATTCAAGTGTGTCAATATTCTATTTTGTATATTAAATGCTATATAATGGGG  3790

Query  6421  ACAAATCTATATTATACTGTGTATGGCATTATTAAGAAGCTTTTTCATTATTTTTTATCA  6480
18665  3791  ACAAATCTATATTATACTGTGTATGGCATTATTAAGAAGCTTTTTCATTATTTTTTATCA  3850

Query  6481  CAGTAATTTTAAAATGTGTAAAAATTAAAACCAGTGACTCCTGTTTAAAAATAAAAGTTG  6540
18665  3851  CAGTAATTTTAAAATGTGTAAAAATTAAAACCAGTGACTCCTGTTTAAAAATAAAAGTTG  3910

Query  6541  TAGTTTTTTATTCATGCTGAATAATAATCTGTAGTTaaaaaaaaaGTGTCTTTTTACCTA  6600
18665  3911  TAGTTTTTTATTCATGCTGAATAATAATCTGTAGTTAAAAAAAAAGTGTCTTTTTACCTA  3970

Query  6601  CGCAGTGAAATGTCAGACTGTAAAACCTTGTGTGGAAATGTTTAACTTTTATTTTTTCAT  6660
18665  3971  CGCAGTGAAATGTCAGACTGTAAAACCTTGTGTGGAAATGTTTAACTTTTATTTTTTCAT  4030

Query  6661  TTAAATTTGCTGTTCTGGTATTACCAAACCACACATTTGTACCGAATTGGCAGTAAATGT  6720
18665  4031  TTAAATTTGCTGTTCTGGTATTACCAAACCACACATTTGTACCGAATTGGCAGTAAATGT  4090

Query  6721  TAGCCATTTACAGCAATGCCAAATATGGAGAAACATCATAATaaaaaaaTCTGCTTTTTC  6780
18665  4091  TAGCCATTTACAGCAATGCCAAATATGGAGAAACATCATAATAAAAAAATCTGCTTTTTC  4150

Query  6781  ATTA  6784
```

FIG. 7E

18665 4151 ATTA 4154

FIG. 7F

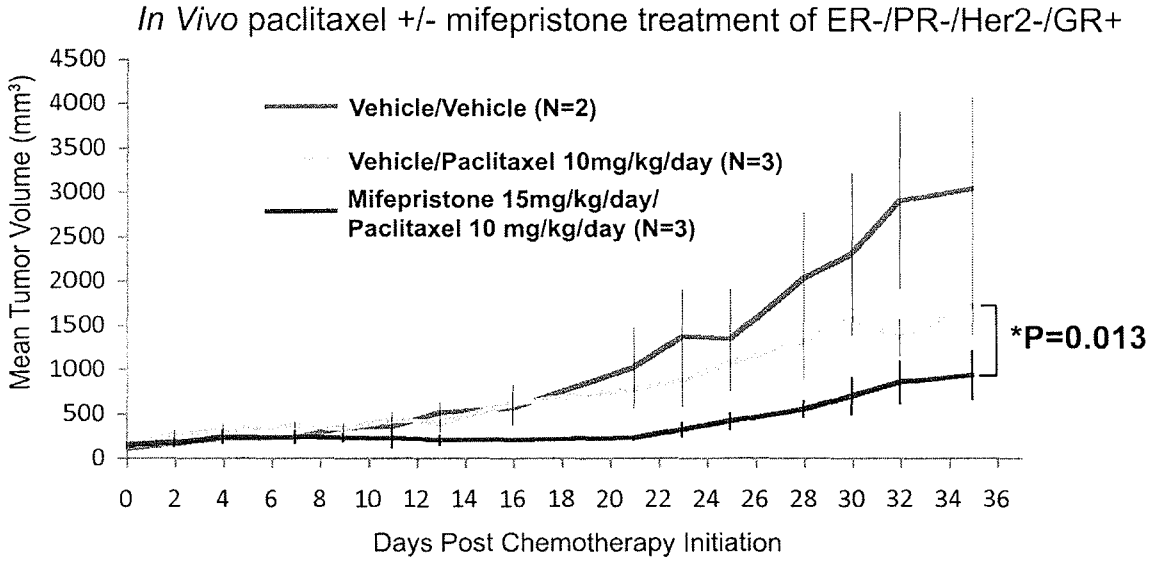
In Vivo paclitaxel +/- mifepristone treatment of ER-/PR-/Her2-/GR+
Mean Tumor Volume (mm³)
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Vehicle/Vehicle (N=2)
Vehicle/Paclitaxel 10mg/kg/day (N=3)
Mifepristone 15mg/kg/day/
Paclitaxel 10 mg/kg/day (N=3)
*P=0.013
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32 34 36
Days Post Chemotherapy Initiation

FIG. 8

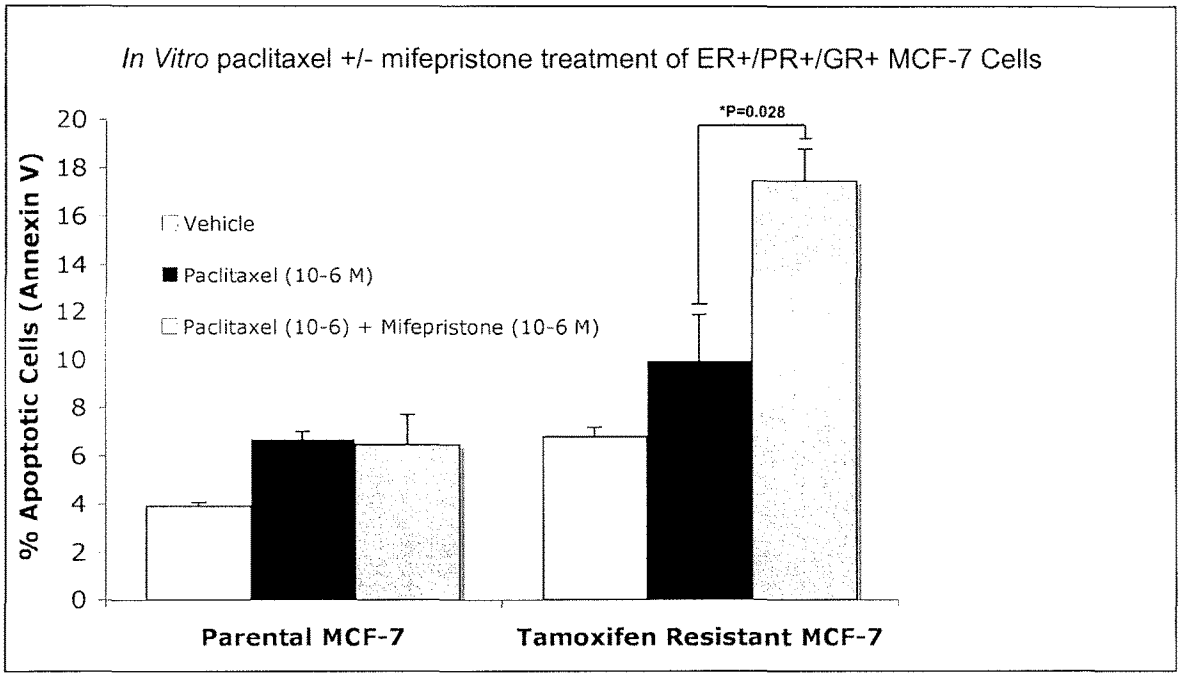
In Vitro paclitaxel +/- mifepristone treatment of ER+/PR+/GR+ MCF-7 Cells
% Apoptotic Cells (Annexin V)
20
18
16
14
12
10
8
6
4
2
0
*P=0.028
Vehicle
Paclitaxel (10-6 M)
Paclitaxel (10-6) + Mifepristone (10-6 M)
Parental MCF-7
Tamoxifen Resistant MCF-7

FIG. 9

METHODS AND COMPOSITIONS RELATED TO GLUCOCORTICOID RECEPTOR ANTAGONISTS AND BREAST CANCER

CROSS-REFERENCES TO RELATED APPLICATIONS

This application is a Continuation of U.S. application Ser. No. 15/448,827, filed Mar. 3, 2017, which is a Continuation of U.S. application Ser. No. 14/296,127, filed Jun. 4, 2014 (now U.S. Pat. No. 9,623,032, issued Apr. 18, 2017), which is a Continuation of U.S. application Ser. No. 14/172,051, filed Feb. 4, 2014 (now U.S. Pat. No. 9,149,485, issued Oct. 6, 2015), which is a Continuation of U.S. application Ser. No. 13/071,363, filed Mar. 24, 2011 (now U.S. Pat. No. 8,710,035, issued Apr. 29, 2014), which claims priority to U.S. Provisional Application No. 61/317,182, filed on Mar. 24, 2010, the disclosures of which are hereby incorporated by reference in their entireties.

STATEMENT AS TO RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH AND DEVELOPMENT

This invention was made with government support under CA089208 awarded by the National Institutes of Health. The government has certain rights in the invention.

REFERENCE TO A "SEQUENCE LISTING," A TABLE, OR A COMPUTER PROGRAM LISTING APPENDIX SUBMITTED ON AN ASCII TEXT FILE

The Sequence Listing written in file "SeqListing096487-1158267.TXT", created on Oct. 7, 2019, 231,396 bytes, machine format IBM-PC, MS-Windows operating system, is hereby incorporated by reference in its entirety for all purposes.

BACKGROUND OF THE INVENTION

I. Field of the Invention

Embodiments of this invention are directed generally to biology and medicine. In certain aspects methods involve determining the prognosis for a breast cancer patient. In other embodiments, there are methods and compositions for treating a breast cancer patient with a glucocorticoid antagonist.

II. Background

There are over 1 million cases of breast cancer per year on a global basis, of which around 0.5 million are in the US, 40,000 are in the UK and nearly 2,000 in Ireland. It is the leading cause of cancer deaths among women (Keen and Davidson, 2003). Although the overall incidence of the disease is increasing within the western world, wider screening and improved treatments have led to a gradual decline in the fatality rate of about 1% per year since 1991. Inheritance of susceptibility genes, such as BRCA1 and BRCA2, account for only 5% of breast cancer cases and the factors responsible for the other 95% remain obscure (Grover and Martin, 2002). In the absence of a strategy to reduce causative agents of breast cancer, early detection remains the best approach to reducing the mortality rate of this disease. It is widely held that breast cancer initiates as the pre-malignant stage of atypical ductal hyperplasia (ADH), progresses into the pre-invasive stage of ductal carcinoma in situ (DCIS), and culminates in the potentially lethal stage of invasive ductal carcinoma (IDC). This linear model of breast cancer progression has been the rationale for the use of detection methods such as mammography in the hope of diagnosing and treating breast cancer at earlier clinical stages (Ma et al., 2003).

As more molecular information is being collated, diseases such as breast cancer are being sub-divided according to genetic signatures linked to patient outcome, providing valuable information for the clinician. Emerging novel technologies in molecular medicine have already demonstrated their power in discriminating between disease sub-types that are not recognizable by traditional pathological criteria (Sorlie et al., 2001) and in identifying specific genetic events involved in cancer progression (Srinivas et al., 2002).

Endocrine therapy is a popular mode of treatment for all stages of breast cancer. A majority of breast cancers belong to the type in which growth is stimulated by the female sex hormones, estrogens and progesterone. Therefore some of the therapies are based on depriving the tumor of the hormone-induced growth stimulus. Some of the current modes of endocrine treatments include blockade of the estrogen receptor with an antiestrogen, e.g. tamoxifen; hormonal ablation by surgery (oophorectomy, adrenalectomy or hypophysectomy), radiotherapy or medically by administration of a luteinizing hormone-releasing hormone analogue (LH-RHa), e.g., goserelin; suppression of estrogen synthesis with aromatase inhibitors, e.g., anastrozole; pharmacological doses of estrogens and progestagens, e.g., megestrol acetate.

Despite recent advances, the challenge of cancer treatment, including breast cancer therapy remains. Progress is limited with respect to the development of specific treatment regimens to clinically distinct tumor types, and to personalize tumor treatment in order to maximize outcome and efficiency. Moreover, a number of patients exhibit chemotherapy resistance.

Mere classification of breast cancers into a few subgroups characterized by low to absent gene expression of the estrogen receptor (ER) alone may not reflect the cellular and molecular heterogeneity of breast cancer, and may not allow the design of treatment strategies maximizing patient response. Once a patient is diagnosed with cancer, such as breast or ovarian cancer, or an individual wants predisposition analysis, there is a strong need for methods that allow the physician to predict the expected course of disease, including the likelihood of cancer recurrence, long-term survival of the patient, and the like, and accordingly select an appropriate treatment option that is effective.

SUMMARY OF THE INVENTION

Embodiments concern methods, compositions, and apparatuses related to assessing, prognosing, and/or treating breast cancer patients. It concerns using information related to glucocorticoid receptor (GR) activity and/or expression in conjunction with information related to estrogen receptor (ER) activity or expression to identify patients with the least favorable prognosis based on current standards of care for breast cancer. Patients with relatively low levels of estrogen receptor expression and relatively high levels of glucocorticoid expression fall into a group of breast cancer patients with the least favorable prognosis (i.e., mortality rate).

Accordingly, methods concern evaluating a patient with breast cancer. Embodiments include evaluating a biological

sample from a patient; evaluating breast cancer cells from a patient; evaluating a biological sample from a breast cancer patient; assessing a breast cancer patient; testing a breast cancer sample or biopsy; testing a breast tumor; prognosing a breast cancer patient; treating a breast cancer patient, particularly a patient with a particular profile related to ER and GR; determining a treatment for a breast cancer patient; altering a treatment plan for a breast cancer patient; reporting prognosis of a breast cancer patient; determining a prognosis score for a breast cancer patient; generating a prognosis score for a breast cancer patient; assessing the risk of mortality of a breast cancer patient generally or within a certain time frame, such as 150 months from end of cancer treatment; generating an ER and GR expression profile for a breast cancer patient; comparing a patient's ER and GR expression profile to a standardized profile; and/or, determining a breast cancer patient has a poor prognosis based on the patient's ER and GR status.

Embodiments also cover apparatuses, kits, and computer readable medium and systems for assessing the level or activity of ER and/or GR in a patient's breast cancer sample and determining a prognosis; and/or treating the patient accordingly. It is specifically contemplated that a breast cancer patient is a human. Accordingly, in human patients, ER refers to an estrogen receptor in a human and GR refers to a glucocorticoid receptor in a human.

Some embodiments include generating an expression profile for glucocorticoid receptor, which means obtaining the level of expression of GR directly or indirectly by measuring or assaying activity or expression. Methods include directly measuring or assaying the level of expression or activity refers to measuring or assaying a sample to determine the level of GR expression (protein or transcript) in the cell. Indirectly obtaining the level of expression includes measuring or assaying expression or activity of a gene or protein that correlates with GR expression or activity. In some embodiments, the level of GR expression can be indirectly obtained by measuring or assaying expression of a GR-responsive gene, which refers to a gene whose expression is affected in a dose-dependent manner by GR expression or activity. Expression refers to either protein expression or RNA (transcript) expression. Methods may involve either type of expression and a variety of assays are well known to those of skill in the art. For example, quantitative PCR may be performed to obtain RNA expression levels. The Affymetrix chip used in the Examples also provides information regarding RNA expression levels. Alternatively, reagents to detect protein expression levels may be employed in embodiments. Methods may involve probes, primers, and/or antibodies that are specific to GR or ER in order to assess expression levels.

In some embodiments, the activity level of GR is measured by assaying the level of GR expression. In additional embodiments, GR expression is GR transcript expression. In other embodiments, GR expression is GR protein expression. As discussed above, in some embodiments, the activity level of GR is measured by assaying the expression level of one or more GR-responsive genes. A GR-responsive gene may be one or more of the following: MCL1, SAP30, DUSP1, SGK1, SMARCA2, PTGDS, TNFRSF9, SFN, LAPTM5, GPSM2, SORT1, DPT, NRP1, ACSL5, BIRC3, NNMT, IGFBP6, PLXNC1, SLC46A3, C14orf139, PIAS1, IDH2, SERPINF1, ERBB2, PECAM1, LBH, ST3GAL5, IL1R1, BIN1, WIPF1, TFPI, FN1, FAM134A, NRIP1, RAC2, SPP1, PHF15, BTN3A2, SESN1, MAP3K5, DPYSL2, SEMA4D, STOM, or MAOA.

In some embodiments, there is a step of assaying or measuring the activity level of glucocorticoid receptor (GR) in a biological sample from the patient containing breast cancer cells. As discussed above, the activity level of GR can be obtained directly or indirectly. It is specifically contemplated that levels of glucocorticoid activity or expression refers to activity or expression of GR α, GR β, or both. Unless specifically stated otherwise, the terms "glucocorticoid receptor" or "GR" refer to both forms. Embodiments discussed with respect to glucocorticoid receptor or GR may also be implemented solely with GRα or solely with GRβ.

Methods may also include obtaining a level of estrogen receptor (ER) expression in breast cancer cells from the patient. The level can be obtained by obtaining the results of an assay that measured the level of ER expression. In some embodiments, the level is obtained by measuring or assaying the level of ER expression.

In some embodiments, the level of estrogen receptor expression in breast cancer cells from patient is obtained by measuring the level of estrogen receptor expression from the biological sample from the patient. In other embodiments, the level is obtained by receiving qualitative and/or quantitative data regarding the level.

In some embodiments, methods include identifying the patient as having or not having a risk factor for cancer recurrence based on the levels of ER and GR expression. Methods may involve categorizing the patient as ER+ or ER− based the level of estrogen receptor expression and a predetermined threshold value for ER expression. The term "ER+" refers to a classification of ER expression that indicates the patient expresses estrogen receptor in breast cancer cells at or above a certain level. The term "ER−" refers to a classification of ER expression that indicates the patient expresses estrogen receptor at a relatively low level in breast cancer cells, meaning at or below a certain level. In embodiments of the invention, that certain level or a predetermined threshold value is at, below, or above 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percentile, or any range derivable therein.

Methods may involve measuring the activity level of glucocorticoid receptor in a biological sample from the patient containing breast cancer cells and measuring the expression level of estrogen receptor in the biological sample.

In certain embodiments, the predetermined threshold value for ER expression identifies a patient as ER+ if the patient's ER expression level is in the 25^{th} percentile or greater compared to a normalized sample. This means the patient may be designated as having a level of ER expression that is at or above 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percentile, or any range derivable therein. It is contemplated that in some cases, a patient may be designated as ER+ if the patient's ER expression level is at or above 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90,

91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or any range derivable therein. The patient may also be referred to as having a normal or high ER expression level. The higher the percentile, the higher the relative expression level.

In embodiments, methods may also involve categorizing the patient as GR+ or GR− based on a predetermined threshold value for GR activity. In some cases, a predetermined threshold value for GR activity is dependent on whether the patient is categorized as ER+ or ER−. Embodiments may involve a predetermined threshold value for GR activity that identifies a patient as GR+ if the patient is ER− and GR activity level is in the 65^{th} percentile or greater compared to a normalized sample. It is contemplated that in some cases, a patient may be designated as GR+ if the patient's GR expression level is at or above 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or any range derivable therein. The threshold value may or may not be dependent on ER expression levels or status. In some embodiments, the threshold value depends on whether the patient is ER− or not. The higher the percentile, the higher the relative expression level.

Methods may involve the use of a normalized sample or control that is based on one or more breast cancer samples that are not from the patient being tested.

In some embodiments, methods involve calculating a prognosis score for the patient based on the levels of ER and/or GR expression. Methods may alternatively or additionally involve reporting a prognosis score or report the levels of ER and/or GR expression. The score or report may contain or reflect raw data regarding expression levels or it may reflect a categorization of the expression levels obtained. A score could indicate the risk factor for mortality, recurrence, and/or both. The score could be a number within a numeric scale in which one end of the scale is most favorable and the other end is the least favorable with respect to a prognosis for breast cancer.

In certain embodiments, methods may involve identifying the patient as having a poor prognosis if the patient is determined to have a glucocorticoid receptor activity level at or above a certain threshold level and a level of estrogen receptor that is at or below a second threshold level. In each case, the threshold levels are specific for each of GR and ER. In certain embodiments, it is contemplated that a GR level in the 65th percentile or above based on breast cancer patients whose are in the 35^{th} percentile or below is indicative of a poor prognosis. In some embodiments, patients with a poor prognosis include a population of breast cancer patients that numbers approximately 10% or less.

Methods also include identifying the patient as having a poor prognosis if the patient is determined to have i) an activity level of glucocorticoid receptor that is higher than the activity level of glucocorticoid receptor in normalized control sample and ii) a expression level of estrogen receptor expression that is lower than the expression level of estrogen receptor in a normalized control sample. Consequently, methods of the invention include prognosing a breast cancer patient. In some cases, a patient is identified as having a relatively good prognosis.

Other embodiments include methods of treating a patient for breast cancer comprising: treating the patient for breast cancer after a biological sample from the patient containing breast cancer cells is analyzed for i) the activity level of glucocorticoid receptor and ii) the expression level of estrogen receptor. A patient may be treated with a different treatment protocol than the patient would have been treated with if the patient's biological sample had not been analyzed. In some embodiments, the patient is categorized as ER− and GR+ based on the activity level of the glucocorticoid receptor and the expression level of estrogen receptor. In some cases, the patient is treated with a more aggressive therapy than the patient would have been treated with if the patient had not been categorized as ER− and GR+. The term "more aggressive" refers to a treatment regimen that may include more drugs or drugs with more severe side effects and/or it may include an increased dosage or increased frequency of drugs. It may also include radiation or a combination of therapies. In some cases, the therapy includes one or more chemotherapeutics and/or biologics. In some embodiments, the patient is treated with a therapy comprising an anti-angiogenic agent. In additional embodiments, the therapy further comprises a chemotherapeutic agent in addition to the anti-angiogenic agent. Embodiments also include administering a glucocorticoid receptor antagonist and/or tyrosine kinase inhibitor.

Embodiments may also include where the patient is treated with more than one type of cancer therapy. This may be after the patient is determined to have a particular prognosis or after the status of the patient's GR and ER expression profile is known. In some embodiments, certain treatments are provided to an ER−/GR+ breast cancer patient who might have otherwise been treated with a less aggressive treatment for breast cancer. In some embodiments, a patient is treated with at least two of the following: radiation, chemotherapy, or a biologic. In particular embodiments, the patient may be treated with a kinase inhibitor and/or anti-angiogenic agent.

Methods may also involve obtaining a biological sample comprising breast cancer cells from the patient and categorizing the patient as i) GR+ or GR− based on the level of glucocorticoid activity assayed in the sample and compared to a predetermined threshold value for GR activity; and ii) ER+ or ER− based on the level of estrogen receptor expression assayed in the sample and compared to a predetermined threshold value for ER expression.

Any method may also include treating the patient for breast cancer, which may include directly administering or providing a cancer therapy. In some embodiments, a practitioner or doctor may prescribe a cancer therapy that the patient administers to herself.

To achieve these methods, a doctor, medical practitioner, or their staff may retrieve a biological sample from a patient for evaluation. The sample may be a biopsy, such as a breast tissue or tumor biopsy. The sample may be analyzed by the practitioner or their staff, or it may be sent to an outside or independent laboratory. The medical practitioner may be cognizant of whether the test is providing information regarding the patient's level of GR and/or ER expression or activity, or the medical practitioner may be aware only that the test indicates directly or indirectly that the test reflects that the patient has a particular prognosis or can be given a particular prognosis score. Furthermore, the practitioner may know the patient's ER or GR status, such as ER+ or ER−, or GR+ or GR−. Alternatively, she may be aware only that the test or assay indicates the patient has a poor prognosis, or the worst prognosis.

Embodiments also concern kits to determine glucocorticoid receptor status in breast cancer cells comprising: (a) one or more reagents for determining expression levels of NR3C1 in a biological sample; and (b) an algorithm and

software encoding the algorithm for calculating a risk factor index from the expression of NR3C1 in a sample and the estrogen receptor status of the breast cancer cells to determine a prognosis or a prognosis score. Kits may also include one or more reagents for determining expression levels of ESR1 in the biological sample to provide estrogen receptor status.

Other embodiments include a computer readable medium having software modules for performing a method comprising the acts of: (a) comparing glucocorticoid receptor data obtained from a patient's breast cancer sample with a reference; and (b) providing an assessment of glucocorticoid receptor status to a physician for use in determining an appropriate therapeutic regimen for a patient. In further embodiments, the computer readable medium further comprises a software module for assessing estrogen receptor status of the patient's breast cancer sample.

Computer systems are also included. In some embodiments, they have a processor, memory, external data storage, input/output mechanisms, a display, for assessing glucocorticoid receptor activity, comprising: (a) a database; (b) logic mechanisms in the computer generating for the database a GR-responsive gene expression reference; and (c) a comparing mechanism in the computer for comparing the GR-responsive gene expression reference to expression data from a patient sample using a comparison model to determine a GR gene expression profile of the sample.

Other embodiments include an internet accessible portal for providing biological information constructed and arranged to execute a computer-implemented method for providing: (a) a comparison of gene expression data of one or more GR-responsive genes in a patient sample with a calculated reporter index; and (b) providing an assessment of GR activity or expression to a physician for use in determining an appropriate therapeutic regime for a patient.

In addition to compiling, collecting and or processing data related to GR status, methods, media and systems may also include the same embodiments with respect to data related to ER status. Such aspects may be instead of or in addition to the aspects related to GR status or data.

Embodiments also include methods of killing breast cancer cells comprising administering to a breast cancer patient an effective amount of a combination of anti-cancer compounds, wherein the anticancer compounds comprise a glucocorticoid receptor antagonist and a chemotherapeutic.

In other embodiments, there are methods for treating breast cancer in a patient comprising administering to the patient an effective amount of glucocorticoid receptor antagonist and a chemotherapeutic.

In further embodiments, methods are provided for treating chemotherapy-insensitive breast cancer cells comprising administering to a breast cancer patient an effective amount of a glucocorticoid receptor antagonist followed by chemotherapy.

Other methods include methods for treating breast cancer in a patient comprising: a) administering radiation or at least a first chemotherapeutic to the patient; b) subsequently administering an effective amount of a glucocorticoid receptor antagonist to the patient; and, c) administering radiation again or at least a second chemotherapeutic to the patient after the glucocorticoid receptor antagonist is administered to the patient.

In some embodiments, there are methods for treating breast cancer in a patient comprising: a) administering an effective amount of a glucocorticoid receptor antagonist to the patient, wherein the patient expresses detectable levels of GR prior to administration of the GR antagonist; b) then administering an effective amount of radiation or at least one chemotherapeutic.

It is contemplated that in methods described herein, breast cancer cells may undergo apoptosis following treatment set forth herein. Moreover, in some embodiments, the combination of a glucocorticoid receptor antagonist and an anticancer agent or compound induces more apoptosis than treatment with just the anticancer treatment alone. In other methods, it is specifically contemplated to exclude treatment with a synthetic glucocorticoid, such as dexamethasone.

Glucocorticoid receptor antagonists are known to those of skill in the art. It refers to a compound or substance that that does not provoke a biological response itself upon binding to the glucocorticoid receptor, but blocks or dampens agonist-mediated responses. Examples include, but are not limited to, beclometasone, betamethasone, budesonide, ciclesonide, flunisolide, fluticasone, mifepristone, mometasone, and triamcinolone. In additional embodiments, the glucocorticoid receptor antagonist has undetectable level or a lower level of activity as a progesterone receptor antagonist. In certain embodiments, the glucocorticoid receptor antagonist has greater than 10-fold, 50-fold, 100-fold, 200-fold, 300-fold, 400-fold, 500-fold, 1000-fold lower binding activity (or any range derivable therein) for another hormone receptor compared to its binding activity for glucocorticoid receptor. In specific embodiments the hormone receptor is estrogen receptor or progesterone receptor.

In some embodiments, a patient had been previously treated with an anti-cancer therapy, such as radiation, chemotherapy, or immunotherapy (or a combination or multiple therapies thereof). In certain embodiments, a first anti-cancer therapy prior to therapy with glucocorticoid receptor antagonist was last administered more than two weeks prior to the glucocorticoid receptor antagonist or its combination with a second anti-cancer therapy. In certain embodiments, this first anti-cancer therapy that does not include a glucocorticoid receptor antagonist was last administered to the breast cancer patient at least 7, 8, 9, 10, 11, 12, 13, 14 days, and/or 1, 2, 3, 4, or 5 weeks, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months prior to treatment with a glucocorticoid receptor antagonist. Treatment methods may be applied to breast cancer or breast cancer cells that are chemo-resistant or breast cancer cells that are not chemo-sensitive. Moreover, treatment may be applied to breast cancer or to breast cancer cells that were previously administered a first apoptosis inducing agent, but were resistant to apoptosis.

In some embodiments, the breast cancer cells are determined to be resistant to apoptosis. In additional embodiments, the breast cancer or the breast cancer cells are determined not to be chemo-sensitive or are determined to be chemo-resistant. This determination may be based on the results of a genetic test or based on information obtained from an assessment of a tumor or the breast cancer after treatment with a first anti-cancer therapy. In specific embodiments, the first anti-cancer therapy is a chemotherapeutic, Herceptin®, radiation, a combination of chemotherapeutics, or a combination of one or more chemotherapeutic agents and Herceptin®.

In additional embodiments, the breast cancer cells express a detectable level of glucocorticoid receptor or its transcript. In some embodiments, the patient is determined to have breast cancer cells that express a detectable level of glucocorticoid receptor or its transcript. This may be determined directly or indirectly.

It is contemplated that breast cancer cells may be treated with a glucocorticoid receptor antagonist regardless of estro-

gen receptor status. Therefore, breast cancer cells may be estrogen receptor-negative (ER−) or estrogen receptor-positive (ER+), accordingly to a standardized and industry accepted test for ER status. In certain embodiments, the breast cancer cells do not express any detectable levels of ER; in other embodiments, ER expression is detectable in the breast cancer cells.

It is contemplated that breast cancer cells may be treated with a glucocorticoid receptor antagonist depending on or regardless of progesterone receptor status. Therefore, breast cancer cells may be progesterone receptor-negative (PR−) or progesterone receptor-positive (PR+), accordingly to a standardized and industry accepted test for ER status. In certain embodiments, the breast cancer cells do not express any detectable levels of PR; in other embodiments, PR expression is detectable in the breast cancer cells.

Methods involve treating breast cancer, particularly a chemo-resistant breast cancer, with a combination of therapies that includes a glucocorticoid receptor antagonist and an anticancer therapy that induces apoptosis (together they may be referred to as a combination of anti-cancer agents or compounds), such as a chemotherapeutic. In some embodiments, the chemotherapeutic is capecitabine, carboplatin, cyclophosphamide (Cytoxan), daunorubicin, docetaxel (Taxotere), doxorubicin (Adriamycin), epirubicin (Ellence), fluorouracil (also called 5-fluorouracil or 5-FU), gemcitabine, eribulin, ixabepilone, methotrexate, mitomycin C, mitoxantrone, paclitaxel (Taxol), thiotepa, vincristine, or vinorelbin, or a combination of these agents. In other embodiments, therapy with a glucocorticoid receptor antagonist is combined Herceptin®, radiation, chemotherapeutic(s) and radiation, a combination of chemotherapeutics, or a combination of one or more chemotherapeutic agents and Herceptin®.

It is contemplated that in some embodiments of the combination therapy the glucocorticoid receptor antagonist is administered within 5, 10, 30, 45, 60 minutes, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, and/or 1, 2, 3, 4, 5, 6, 7 days, or any combination thereof within administration of at least one or the combination of the anti-cancer agents or compounds. In specific embodiments, the glucocorticoid receptor antagonist is administered within 2 hours, 12 hours or 24 hours of administration of a anticancer agent or compound (or a combination of such agents or compounds).

It is specifically contemplated that treatment may continue or be repeated. In some embodiments, once treated with the combination of a glucocorticoid receptor antagonist and at least one anticancer agent or compound, all or part of the treatment may be repeated alone or in combination with a different anticancer agent or compound.

In certain embodiments, the glucocorticoid receptor antagonist is administered prior to as the other agent or therapy included in the combination therapy. In certain embodiments, the glucocorticoid receptor antagonist is administered 5, 10, 30, 45, 60 minutes, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, and/or 1, 2, 3, 4, 5, 6, 7 days, or any combination thereof prior to administration of at least one or the combination of the anti-cancer agents or compounds. It is specifically contemplated that in some embodiments, the glucocorticoid receptor antagonist is given prior to administration of the anticancer agent or compound but that the glucocorticoid receptor antagonist is also given concurrently with or after administration of the initial or a subsequent dose of the anticancer agent or compound. As discussed throughout, the anticancer agent or compound may be in a combination of such agents or compounds. In certain embodiments, the glucocorticoid receptor antagonist is administered up to three days prior to administering the anticancer agent or compound.

Additionally or alternatively, the glucocorticoid receptor antagonist is administered after administration of the other agent or therapy included in the combination therapy. In certain embodiments, the glucocorticoid receptor antagonist is administered 5, 10, 30, 45, 60 minutes, and/or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 hours, and/or 1, 2, 3, 4, 5, 6, 7 days, or any combination thereof after administration of at least one or the combination of the anti-cancer agents or compounds. It is specifically contemplated that in some embodiments, the glucocorticoid receptor antagonist is given after to administration of the anticancer agent or compound; such administration may be repeated. As discussed throughout, the anticancer agent or compound may be in a combination of such agents or compounds. In certain embodiments, the glucocorticoid receptor antagonist is administered up to three days after administering the anticancer agent or compound.

In certain embodiments, the breast cancer is an unresectable breast cancer. In further embodiments, the breast cancer is inflammatory breast cancer.

It is specifically contemplated that in some methods, dexamethasone has not been administered to the patient within 24 hours of administration of the glucocorticoid receptor antagonist.

Compositions are contemplated to include a glucocorticoid receptor antagonist and any other anticancer compound discussed herein, such a Herceptin or one or more chemotherapeutic compounds. In some embodiments, the composition is in a pharmaceutically acceptable formulation.

Use of the one or more compositions may be employed based on methods described herein. Other embodiments are discussed throughout this application. Any embodiment discussed with respect to one aspect of the invention applies to other aspects of the invention as well and vice versa. The embodiments in the Example section are understood to be embodiments o that are applicable to all aspects of the technology described herein.

"Cancer prognosis" generally refers to a forecast or prediction of the probable course or outcome of the cancer. As used herein, cancer prognosis includes the forecast or prediction of any one or more of the following: duration of survival of a patient susceptible to or diagnosed with a cancer, duration of recurrence-free survival, duration of progression free survival of a patient susceptible to or diagnosed with a cancer, response rate in a group of patients susceptible to or diagnosed with a cancer, and/or duration of response in a patient or a group of patients susceptible to or diagnosed with a cancer.

In certain aspects, prognosis is an estimation of the likelihood of metastasis free survival of said patient over a predetermined period of time, e.g., over a period of 5 years.

In further aspects, prognosis is an estimation of the likelihood of death of disease of said patient over a predetermined period of time, e.g., over a period of 5 years.

The term "recurrence" refers to the detection of breast cancer in form of metastatic spread of tumor cells, local recurrence, contralateral recurrence or recurrence of breast cancer at any site of the body of the patient after breast cancer had been substantially undetectable or responsive to treatments.

As used herein, "prognostic for cancer" means providing a forecast or prediction of the probable course or outcome of

the cancer. In some embodiments, "prognostic for cancer" comprises providing the forecast or prediction of (prognostic for) any one or more of the following: duration of survival of a patient susceptible to or diagnosed with a cancer, duration of recurrence-free survival, duration of progression free survival of a patient susceptible to or diagnosed with a cancer, response rate in a group of patients susceptible to or diagnosed with a cancer, and/or duration of response in a patient or a group of patients susceptible to or diagnosed with a cancer.

By "gene" is meant any polynucleotide sequence or portion thereof with a functional role in encoding or transcribing a protein or regulating other gene expression. The gene may consist of all the nucleic acids responsible for encoding a functional protein or only a portion of the nucleic acids responsible for encoding or expressing a protein. The polynucleotide sequence may contain a genetic abnormality within exons, introns, initiation or termination regions, promoter sequences, other regulatory sequences or unique adjacent regions to the gene.

As used herein, "treatment" or "therapy" is an approach for obtaining beneficial or desired clinical results. This includes: reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and/or stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and/or stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder, shrinking the size of the tumor, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of patients.

The term "therapeutically effective amount" refers to an amount of the drug that may reduce the number of cancer cells; reduce the tumor size; inhibit (i.e., slow to some extent and preferably stop) cancer cell infiltration into peripheral organs; inhibit (i.e., slow to some extent and preferably stop) tumor metastasis; inhibit, to some extent, tumor growth; and/or relieve to some extent one or more of the symptoms associated with the disorder. To the extent the drug may prevent growth and/or kill existing cancer cells, it may be cytostatic and/or cytotoxic. For cancer therapy, efficacy in vivo can, for example, be measured by assessing the duration of survival, time to disease progression (TTP), the response rates (RR), duration of response, and/or quality of life.

The terms "overexpress", "overexpression", "overexpressed", "up-regulate", or "up-regulated" interchangeably refer to a biomarker that is transcribed or translated at a detectably greater level, usually in a cancer cell, in comparison to a non-cancer cell or cancer cell that is not associated with the worst or poorest prognosis. The term includes overexpression due to transcription, post transcriptional processing, translation, post-translational processing, cellular localization, and/or RNA and protein stability, as compared to a non-cancer cell or cancer cell that is not associated with the worst or poorest prognosis. Overexpression can be detected using conventional techniques for detecting mRNA (i.e., RT-PCR, PCR, hybridization) or proteins (i.e., ELISA, immunohistochemical techniques, mass spectroscopy). Overexpression can be 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or more in comparison to a normal cell or cancer cell that is not associated with the worst or poorest prognosis. In certain instances, overexpression is 1-fold, 2-fold, 3-fold, 4-fold 5, 6, 7, 8, 9, 10, or 15-fold or more higher levels of transcription or translation in comparison to a non-cancer cell or cancer cell that is not associated with the worst or poorest prognosis.

"Biological sample" includes sections of tissues such as biopsy and autopsy samples, and frozen sections taken for histologic purposes. Such samples include breast cancer tissues, cultured cells, e.g., primary cultures, explants, and transformed cells. A biological sample is typically obtained from a mammal, such as a primate, e.g., human.

A "biopsy" refers to the process of removing a tissue sample for diagnostic or prognostic evaluation, and to the tissue specimen itself. Any biopsy technique known in the art can be applied to the diagnostic and prognostic methods of the present invention. The biopsy technique applied will depend on the tissue type to be evaluated (e.g., breast), the size and type of the tumor, among other factors. Representative biopsy techniques include, but are not limited to, excisional biopsy, incisional biopsy, needle biopsy, and surgical biopsy. An "excisional biopsy" refers to the removal of an entire tumor mass with a small margin of normal tissue surrounding it. An "incisional biopsy" refers to the removal of a wedge of tissue that includes a cross-sectional diameter of the tumor. A diagnosis or prognosis made by endoscopy or fluoroscopy can require a "core-needle biopsy", or a "fine-needle aspiration biopsy" which generally obtains a suspension of cells from within a target tissue. Biopsy techniques are discussed, for example, in Harrison's Principles of Internal Medicine, 2005. Obtaining a biopsy includes both direct and indirect methods, including obtaining the biopsy from the patient or obtaining the biopsy sample after it is removed from the patient.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

Throughout this application, the term "about" is used to indicate that a value includes the standard deviation of error for the device or method being employed to determine the value.

The use of the term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." It is also contemplated that anything listed using the term "or" may also be specifically excluded.

As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

Other objects, features and advantages of the present invention will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples, while indicating specific embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this detailed description.

DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to further demonstrate certain

aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

FIG. **1**. Primary human breast ductal epithelium, DCIS (60%) in vasive human cancers ('30-40%)exhibit significant glucocorticoid receptor expression.

FIG. **2**. Unsupervised cluster analysis identifies GR target gene signature (Sig+) vs Sig− tumors (n=68 genes) A GR-regulated gene expression set from MCF10A-Myc (ER−/GR+) cells treated+/− Dex from 30 m-24 h was used to perform a two dimensional unsupervised clustering analysis on the NKI-295 early breast cancer gene expression data set (n=2034 starting genes). GR-regulated genes (n=68) that separate these tumors into two groups (GRsig+=Red and GRsig−=Green) are shown in rows while each column represents a patient. Several EMT genes (e.g. Snail) and known anti-apoptotic genes are included.

FIG. **3**. NR3C1 expression correlates with GR signature gene expression. The GRsig+ vs. GRsig− tumor designations correlate with higher NR3C1 vs. lower expression, respectively. For ESR1+ tumors (orange) the P<.00001 and for ESR1− tumors (green) p=.7 (t test). Error bars are +/−SD.

FIG. **4**. RFS of GR gene expression signature. The GR signature predicts a differential prognosis for ESR1+ patients and ESR1− pts with respect to GR-signature expression. ESR1−/GR+ signature patients have the worst prognosis.

FIG. **5**. Meta-analysis of NR3C1 expression and RFS.

FIG. **6**. Common genes differentially expressed in ESR1− and NR3C1+/− tumors, ChIP-seq and gene expression in Dex-treated MCF10A-Myc cells.

FIG. **7**A-F. Schematic of glucocorticoid receptor (GR) isoforms. GR alpha=SEQ ID NO:47; GR beta=SEQ ID NO:48

FIG. **8**. Administration of mifepristone increases MDA-MB-231 tumor susceptibility to paclitaxel treatment in vivo.

FIG. **9**. Mifepristone pretreatment increases tamoxifen-resistant MCF-7 (T-R-MCF-7), but not parental MCF-7 cell susceptibility to paclitaxel in vitro.

DETAILED DESCRIPTION OF THE INVENTION

Glucocorticoid receptor (GR) activation initiates a potent cell survival signal in ER− breast cancer models. However, GR activity has not been previously examined in primary human breast cancers. Because anti-apoptotic signaling is believed to be an important determinant of breast cancer viability and relapse, the inventors contemplate that early stage primary human breast cancer demonstrates a correlation between high GR (NR3C1) and GR-mediated gene expression and cancer recurrence.

The Dutch NKI 295 data set was examined and the inventors determined that a gene expression signature of 68 GR-regulated genes (based on in vitro data) could cluster patients into different groups with differential outcome. In addition, it was found that GR-mediated gene expression correlated with NR3C1 expression levels. The inventors examined NR3C1 tumor expression in a much larger meta-dataset and again found that ER−/GR (NR3C1)+ patients did the worst. Moreover, key cell survival genes identified as GR gene targets from ChIP-seq experiments were differentially expressed.

I. Hormone Receptor Status of Breast Cancer

Intracellular receptors (IRs) form a class of structurally-related genetic regulators scientists have named "ligand dependent transcription factors" (R. M. Evans, Science, 240:889, 1988). Steroid receptors are a recognized subset of the IRs, including androgen receptor (AR), progesterone receptor (PR), estrogen receptor (ER), glucocorticoid receptor (GR), and mineralocorticoid receptor (MR). Regulation of a gene by such factors requires both the IR itself and a corresponding ligand, which has the ability to selectively bind to the IR in a way that affects gene transcription.

Naturally occurring as well as synthetic steroidal glucocorticoids (e.g., cortisol, cortisone, prednisolone, dexamethasone) have been widely used for over fifty years for the treatment of acute and chronic inflammatory and immune disorders. In particular, glucocorticoids have been prescribed for the treatment of rheumatoid arthritis, osteoarthritis, rheumatic fever, asthma, allergic rhinitis, systemic lupus erythematosus, chronic obstructive pulmonary disease, Crohn's disease, inflammatory bowel disease, and ulcerative colitis. However, the use of glucocorticoids is often associated with severe and sometimes irreversible side effects such as bone loss/osteoporosis, hyperglycemia, diabetes mellitus, hypertension, glaucoma, muscle atrophy, Cushing's syndrome, and psychosis.

Glucocorticoids exert their pharmacological effects by regulating gene transcription after the formation of a complex with the glucocorticoid receptor (GR). GR-glucocorticoid complex affects gene transcription by translocating to the nucleus after binding of the glucocorticoid where it acts as a dimer in binding to DNA glucocorticoid hormone response elements (GREs) in the promoter regions of particular genes. The GR-glucocorticoid/GRE complex then, in turn, activates (transactivation) or inhibits transcription of proximally located genes. Conversely, the GR-glucocorticoid complex may negatively regulate gene transcription by a process that does not involve binding to DNA. In this process, termed transrepression, following binding of the glucocorticoid, the complexed GR enters the nucleus where it acts as a monomer to directly interact (via protein-protein interaction) with other transcription factors, repressing their ability to induce gene transcription and thus protein expression.

Estrogen, mediated through the estrogen receptor (ER), plays a major role in regulating the growth and differentiation of normal breast epithelium (Pike et al. Epidemiologic Reviews (1993) 15(1):17-35; Henderson et al. Cancer Res. (1988) 48:246-253). It stimulates cell proliferation and regulates the expression of other genes, including the progesterone receptor (PgR). PgR then mediates the mitogenic effect of progesterone, further stimulating proliferation (Pike et al., 1993; Henderson et al., 1988). The molecular differences between estrogen receptor ("ER") negative and ER positive tumors are significant in light of clinical observations which indicate that the nature and biological behavior of ER positive and ER negative tumors are distinct even in the absence of hormonal therapy. For example, ER negative cancers tend to recur sooner and show a different rate of recurrence in distant organ sites compared to ER positive tumors. Clinical observations and molecular profiling data suggest that tumors not expressing both ER and PgR represent a different clinical entity in terms of chemotherapy responsiveness. (Colleoni et al., Annals of Oncology 11(8): 1057 (2000)). Thus, ER negative and ER positive breast cancers are two distinct disease entities rather than phenotypic variations of the same disease.

Relatively increased expression of these genes in primary ER-negative human breast tumors is associated with high GR expression and with an earlier relapse in ER-negative breast cancer patients (described herein). Activation of the

glucocorticoid receptor (GR) in epithelial cells has been shown to initiate an anti-apoptotic (i.e., cell survival) signaling pathway that prevents breast (Wu et al, 2004) and ovarian cancer (Melhem et al, 2009) cell death in vitro and in vivo (Pang et al, 2006). Blocking or antagonizing GR activation with a GR antagonist such as mifepristone reverses cell survival signaling pathways initiated by the GR (Moran et al., 2000). Other GR antagonists (e.g., dexamethasone oxetanone) also reverse GR-mediated cell survival and potentiate apoptosis in response to cell stressors such as growth factor withdrawal (Mikosz et al, 2001). The mechanism(s) whereby GR activation protects from cell death includes the transcriptional upregulation of genes encoding anti-apoptotic proteins such as SGK1, MKP1, MCL1, and BIRC3. However, experiments with a glucocorticoid receptor antagonist, RU486, in conjunction with dexamethasone did not increase the number of apoptotic cells induced by paclitaxel, compared to paclitaxel alone (Wu et al., 2004).

II. Biomarkers and Evaluating Levels of Biomarkers

Biomarkers for prognosing human breast cancer patients have been identified. They include estrogen receptor (ER) in combination with the activity of the glucocorticoid receptor (GR) activity. It is contemplated that these biomarkers may be evaluated based on their gene products. In some embodiments, the gene product is the RNA transcript. In other embodiments, the gene product is the protein expressed by the RNA transcript. In still another embodiment is the evaluation of surrogate genes or gene targets of ER, GR, or ER and GR.

In certain aspects a meta-analysis of expression or activity can be performed. In statistics, a meta-analysis combines the results of several studies that address a set of related research hypotheses. This is normally done by identification of a common measure of effect size, which is modeled using a form of meta-regression. Generally, three types of models can be distinguished in the literature on meta-analysis: simple regression, fixed effects meta-regression and random effects meta-regression. Resulting overall averages when controlling for study characteristics can be considered meta-effect sizes, which are more powerful estimates of the true effect size than those derived in a single study under a given single set of assumptions and conditions. A meta-gene expression value, in this context, is to be understood as being the median of the normalized expression of a marker gene or activity. Normalization of the expression of a marker gene is preferably achieved by dividing the expression level of the individual marker gene to be normalized by the respective individual median expression of this marker genes, wherein said median expression is preferably calculated from multiple measurements of the respective gene in a sufficiently large cohort of test individuals. The test cohort preferably comprises at least 3, 10, 100, 200, 1000 individuals or more including all values and ranges thereof. Dataset-specific bias can be removed or minimized allowing multiple datasets to be combined for meta-analyses (See Sims et al. BMC Medical Genomics (1:42), 1-14, 2008, which is incorporated herein by reference in its entirety).

The calculation of a meta-gene expression value is performed by: (i) determining the gene expression value of at least two, preferably more genes (ii) "normalizing" the gene expression value of each individual gene by dividing the expression value with a coefficient which is approximately the median expression value of the respective gene in a representative breast cancer cohort (iii) calculating the median of the group of normalized gene expression values.

A gene shall be understood to be specifically expressed in a certain cell type if the expression level of said gene in said cell type is at least 2-fold, 5-fold, 10-fold, 100-fold, 1000-fold, or 10000-fold higher than in a reference cell type, or in a mixture of reference cell types. Reference cell types include non-cancerous breast tissue cells or a heterogenous population of breast cancers.

In certain algorithms a suitable threshold level is first determined for a marker gene. The suitable threshold level can be determined from measurements of the marker gene expression in multiple individuals from a test cohort. The median expression of the marker gene in said multiple expression measurements is taken as the suitable threshold value.

Comparison of multiple marker genes with a threshold level can be performed as follows:

1. The individual marker genes are compared to their respective threshold levels.

2. The number of marker genes, the expression level of which is above their respective threshold level, is determined.

3. If a marker genes is expressed above its respective threshold level, then the expression level of the marker gene is taken to be "above the threshold level".

"A sufficiently large number", in this context, means preferably 30%, 50%, 80%, 90%, or 95% of the marker genes used.

In certain aspects, the determination of expression levels is on a gene chip, such as an Affymetrix™ gene chip.

In another aspect, the determination of expression levels is done by kinetic real time PCR.

In certain aspects, the methods can relate to a system for performing such methods, the system comprising (a) apparatus or device for storing data on the ER or nodal status of the patient; (b) apparatus or device for determining the expression level of at least one marker gene or activity; (c) apparatus or device for comparing the expression level of the first marker gene or activity with a predetermined first threshold value; (d) apparatus or device for determining the expression level of at least one second marker gene or activity; and (e) computing apparatus or device programmed to provide a unfavorable or poor prognosis if the data indicates a negative ER status and an increased or decreased expression level of said first marker gene or activity (e.g., GR expression or activity) with the predetermined first threshold value and, alternatively, the expression level of said second marker gene is above or below a predetermined second threshold level.

The person skilled in the art readily appreciates that an unfavorable or poor prognosis can be given if the expression level of the first marker gene with the predetermined first threshold value indicates a tumor that is likely to recur or not respond well to standard therapies.

The expression patterns can also be compared by using one or more ratios between the expression levels of different breast cancer biomarkers. Other suitable measures or indicators can also be employed for assessing the relationship or difference between different expression patterns.

The GR nucleic acid and protein sequences are provided in GenBank accession number AY436590. The ER nucleic acid and protein sequences are provided in GenBank accession number NG_008493. The content of all of these GenBank Accession numbers is specifically incorporated herein by reference as of the filing date of this application.

The following biomarkers are provided for implementation with embodiments discussed herein. All of them designate nucleic acid sequences for the particular gene identifier. Nucleic acid sequences related to these gene designation can be found in the Genbank sequence data-

bases. Additional biomarkers include the MCL1, SAP30, DUSP1, SGK1, SMARCA2, PTGDS, TNFRSF9, SFN, LAPTM5, GPSM2, SORT1, DPT, NRP1, ACSL5, BIRC3, NNMT, IGFBP6, PLXNC1, SLC46A3, C14orf139, PIAS1, IDH2, SERPINF1, ERBB2, PECAM1, LBH, ST3GAL5, IL1R1, BIN1, WIPF1, TFPI, FN1, FAM134A, NRIP1, RAC2, SPP1, PHF15, BTN3A2, SESN1, MAP3K5, DPYSL2, SEMA4D, STOM, and MAOA genes.

One or more of the biomarkers can be used to prognose a human patient with breast cancer. The expression pattern of these biomarkers in breast cancer cells may be used to evaluate a patient to determine whether they are likely to respond to standard chemotherapy, likely not to respond to standard chemotherapy, or likely to relapse after standard chemotherapy.

The expression levels of breast cancer biomarkers can be compared to reference expression levels using various methods. These reference levels can be determined using expression levels of a reference based on all breast cancer patients or all breast cancer patients determined to be ER+and/or ER−. Alternatively, it can be based on an internal reference such as a gene that is expressed in all cells. In some embodiments, the reference is a gene expressed in breast cancer cells at a higher level than any biomarker. Any comparison can be performed using the fold change or the absolute difference between the expression levels to be compared. One or more breast cancer biomarkers can be used in the comparison. It is contemplated that 1, 2, 3, 4, 5, 6, 7, 8, and/or 9 biomarkers may be compared to each other and/or to a reference that is internal or external. A person of ordinary skill in the art would know how to do such comparisons.

Comparisons or results from comparisons may reveal or be expressed as x-fold increase or decrease in expression relative to a standard or relative to another biomarker or relative to the same biomarker but in a different class of prognosis. In some embodiments, patients with a poor prognosis have a relatively high level of expression (overexpression) or relatively low level of expression (underexpression) when compared to patients with a better or favorable prognosis, or vice versa.

Fold increases or decreases may be, be at least, or be at most 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-, 10-, 11-, 12-, 13-, 14-, 15-, 16-, 17-, 18-, 19-, 20-, 25-, 30-, 35-, 40-, 45-, 50-, 55-, 60-, 65-, 70-, 75-, 80-, 85-, 90-, 95-, 100- or more, or any range derivable therein. Alternatively, differences in expression may be expressed as a percent decrease or increase, such as at least or at most 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900, 1000% difference, or any range derivable therein.

Other ways to express relative expression levels are by normalized or relative numbers such as 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.02, 0.03. 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7. 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 8.0, 8.1, 8.2, 8.3, 8.4, 8.5, 8.6, 8.7, 8.8, 8.9, 9.0, 9.1, 9.2, 9.3, 9.4, 9.5, 9.6, 9.7, 9.8, 9.9, 10.0, or any range derivable therein.

Algorithms, such as the weighted voting programs, can be used to facilitate the evaluation of biomarker levels. In addition, other clinical evidence can be combined with the biomarker-based test to reduce the risk of false evaluations. Other cytogenetic evaluations may be considered in some embodiments of the invention.

Any biological sample from the patient that contains breast cancer cells may be used to evaluate the expression pattern of any biomarker discussed herein. In some embodiments, a biological sample from a breast tumor is used. Evaluation of the sample may involve, though it need not involve, panning (enriching) for cancer cells or isolating the cancer cells.

A. Nucleic Acids

Screening methods based on differentially expressed gene products are well known in the art. In accordance with one aspect of the present invention, the differential expression patterns of breast cancer biomarkers can be determined by measuring the levels of RNA transcripts of these genes, or genes whose expression is modulated by the these genes, in the patient's breast cancer cells. Suitable methods for this purpose include, but are not limited to, RT-PCR, Northern Blot, in situ hybridization, Southern Blot, slot-blotting, nuclease protection assay and oligonucleotide arrays.

In certain aspects, RNA isolated from breast cancer cells can be amplified to cDNA or cRNA before detection and/or quantitation. The isolated RNA can be either total RNA or mRNA. The RNA amplification can be specific or non-specific. Suitable amplification methods include, but are not limited to, reverse transcriptase PCR, isothermal amplification, ligase chain reaction, and Qbeta replicase. The amplified nucleic acid products can be detected and/or quantitated through hybridization to labeled probes. In some embodiments, detection may involve fluorescence resonance energy transfer (FRET) or some other kind of quantum dots.

Amplification primers or hybridization probes for a breast cancer biomarker can be prepared from the gene sequence or obtained through commercial sources, such as Affymatrix. In certain embodiments the gene sequence is identical or complementary to at least 8 contiguous nucleotides of the coding sequence.

Sequences suitable for making probes/primers for the detection of their corresponding breast cancer biomarkers include those that are identical or complementary to all or part of genes or SEQ ID NOs described herein. These sequences are all nucleic acid sequences of breast cancer biomarkers.

The use of a probe or primer of between 13 and 100 nucleotides, preferably between 17 and 100 nucleotides in length, or in some aspects of the invention up to 1-2 kilobases or more in length, allows the formation of a duplex molecule that is both stable and selective. Molecules having complementary sequences over contiguous stretches greater than 20 bases in length are generally preferred, to increase stability and/or selectivity of the hybrid molecules obtained. One will generally prefer to design nucleic acid molecules for hybridization having one or more complementary sequences of 20 to 30 nucleotides, or even longer where desired. Such fragments may be readily prepared, for example, by directly synthesizing the fragment by chemical means or by introducing selected sequences into recombinant vectors for recombinant production.

In one embodiment, each probe/primer comprises at least 15 nucleotides. For instance, each probe can comprise at least or at most 20, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 400 or more nucleotides (or any range derivable therein). They may have these lengths and have a sequence that is identical or complementary to a gene or SEQ ID NO described herein. Preferably, each probe/primer has relatively high sequence complexity and does not have any ambiguous residue (undetermined "n" residues).

The probes/primers preferably can hybridize to the target gene, including its RNA transcripts, under stringent or highly stringent conditions. In some embodiments, because each of the biomarkers has more than one human sequence, it is contemplated that probes and primers may be designed for use with each on of these sequences. For example, inosine is a nucleotide frequently used in probes or primers to hybridize to more than one sequence. It is contemplated that probes or primers may have inosine or other design implementations that accommodate recognition of more than one human sequence for a particular biomarker.

For applications requiring high selectivity, one will typically desire to employ relatively high stringency conditions to form the hybrids. For example, relatively low salt and/or high temperature conditions, such as provided by about 0.02 M to about 0.10 M NaCl at temperatures of about 50° C. to about 70° C. Such high stringency conditions tolerate little, if any, mismatch between the probe or primers and the template or target strand and would be particularly suitable for isolating specific genes or for detecting specific mRNA transcripts. It is generally appreciated that conditions can be rendered more stringent by the addition of increasing amounts of formamide.

In another embodiment, the probes/primers for a gene are selected from regions which significantly diverge from the sequences of other genes. Such regions can be determined by checking the probe/primer sequences against a human genome sequence database, such as the Entrez database at the NCBI. One algorithm suitable for this purpose is the BLAST algorithm. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length W in the query sequence, which either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighborhood word score threshold. These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are then extended in both directions along each sequence to increase the cumulative alignment score. Cumulative scores are calculated using, for nucleotide sequences, the parameters M (reward score for a pair of matching residues; always >0) and N (penalty score for mismatching residues; always <0). The BLAST algorithm parameters W, T, and X determine the sensitivity and speed of the alignment. These parameters can be adjusted for different purposes, as appreciated by one of ordinary skill in the art.

In one embodiment, quantitative RT-PCR (such as TaqMan, ABI) is used for detecting and comparing the levels of RNA transcripts in breast cancer samples. Quantitative RT-PCR involves reverse transcription (RT) of RNA to cDNA followed by relative quantitative PCR (RT-PCR). The concentration of the target DNA in the linear portion of the PCR process is proportional to the starting concentration of the target before the PCR was begun. By determining the concentration of the PCR products of the target DNA in PCR reactions that have completed the same number of cycles and are in their linear ranges, it is possible to determine the relative concentrations of the specific target sequence in the original DNA mixture. If the DNA mixtures are cDNAs synthesized from RNAs isolated from different tissues or cells, the relative abundances of the specific mRNA from which the target sequence was derived may be determined for the respective tissues or cells. This direct proportionality between the concentration of the PCR products and the relative mRNA abundances is true in the linear range portion of the PCR reaction. The final concentration of the target DNA in the plateau portion of the curve is determined by the availability of reagents in the reaction mix and is independent of the original concentration of target DNA. Therefore, the sampling and quantifying of the amplified PCR products preferably are carried out when the PCR reactions are in the linear portion of their curves. In addition, relative concentrations of the amplifiable cDNAs preferably are normalized to some independent standard, which may be based on either internally existing RNA species or externally introduced RNA species. The abundance of a particular mRNA species may also be determined relative to the average abundance of all mRNA species in the sample.

In one embodiment, the PCR amplification utilizes one or more internal PCR standards. The internal standard may be an abundant housekeeping gene in the cell or it can specifically be GAPDH, GUSB and β-2 microglobulin. These standards may be used to normalize expression levels so that the expression levels of different gene products can be compared directly. A person of ordinary skill in the art would know how to use an internal standard to normalize expression levels.

A problem inherent in clinical samples is that they are of variable quantity and/or quality. This problem can be overcome if the RT-PCR is performed as a relative quantitative RT-PCR with an internal standard in which the internal standard is an amplifiable cDNA fragment that is similar or larger than the target cDNA fragment and in which the abundance of the mRNA encoding the internal standard is roughly 5-100 fold higher than the mRNA encoding the target. This assay measures relative abundance, not absolute abundance of the respective mRNA species.

In another embodiment, the relative quantitative RT-PCR uses an external standard protocol. Under this protocol, the PCR products are sampled in the linear portion of their amplification curves. The number of PCR cycles that are optimal for sampling can be empirically determined for each target cDNA fragment. In addition, the reverse transcriptase products of each RNA population isolated from the various samples can be normalized for equal concentrations of amplifiable cDNAs.

Nucleic acid arrays can also be used to detect and compare the differential expression patterns of breast cancer biomarkers in breast cancer cells. The probes suitable for detecting the corresponding breast cancer biomarkers can be stably attached to known discrete regions on a solid substrate. As used herein, a probe is "stably attached" to a discrete region if the probe maintains its position relative to the discrete region during the hybridization and the subsequent washes. Construction of nucleic acid arrays is well known in the art. Suitable substrates for making polynucleotide arrays include, but are not limited to, membranes, films, plastics and quartz wafers.

A nucleic acid array of the present invention can comprise at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 60, 70, 80, 90, 100, 150, 200, 250 or more different polynucleotide probes, which may hybridize to different and/or the same biomarkers. Multiple probes for the same gene can be used on a single nucleic acid array. Probes for other disease genes can also be included in the nucleic acid array. The probe density on the array can be in any range. In some embodiments, the density may be 50, 100, 200, 300, 400, 500 or more probes/cm^2.

Specifically contemplated by the present inventors are chip-based nucleic acid technologies such as those described by Hacia et al. (1996) and Shoemaker et al. (1996). Briefly, these techniques involve quantitative methods for analyzing large numbers of genes rapidly and accurately. By tagging

genes with oligonucleotides or using fixed probe arrays, one can employ chip technology to segregate target molecules as high density arrays and screen these molecules on the basis of hybridization (see also, Pease et al., 1994; and Fodor et al, 1991). It is contemplated that this technology may be used in conjunction with evaluating the expression level of one or more breast cancer biomarkers with respect to diagnostic, prognostic, and treatment methods of the invention.

The present invention may involve the use of arrays or data generated from an array. Data may be readily available. Moreover, an array may be prepared in order to generate data that may then be used in correlation studies.

An array generally refers to ordered macroarrays or microarrays of nucleic acid molecules (probes) that are fully or nearly complementary or identical to a plurality of mRNA molecules or cDNA molecules and that are positioned on a support material in a spatially separated organization. Macroarrays are typically sheets of nitrocellulose or nylon upon which probes have been spotted. Microarrays position the nucleic acid probes more densely such that up to 10,000 nucleic acid molecules can be fit into a region typically 1 to 4 square centimeters. Microarrays can be fabricated by spotting nucleic acid molecules, e.g., genes, oligonucleotides, etc., onto substrates or fabricating oligonucleotide sequences in situ on a substrate. Spotted or fabricated nucleic acid molecules can be applied in a high density matrix pattern of up to about 30 non-identical nucleic acid molecules per square centimeter or higher, e.g. up to about 100 or even 1000 per square centimeter. Microarrays typically use coated glass as the solid support, in contrast to the nitrocellulose-based material of filter arrays. By having an ordered array of complementing nucleic acid samples, the position of each sample can be tracked and linked to the original sample. A variety of different array devices in which a plurality of distinct nucleic acid probes are stably associated with the surface of a solid support are known to those of skill in the art. Useful substrates for arrays include nylon, glass and silicon. Such arrays may vary in a number of different ways, including average probe length, sequence or types of probes, nature of bond between the probe and the array surface, e.g. covalent or non-covalent, and the like. The labeling and screening methods of the present invention and the arrays are not limited in its utility with respect to any parameter except that the probes detect expression levels; consequently, methods and compositions may be used with a variety of different types of genes.

Representative methods and apparatus for preparing a microarray have been described, for example, in U.S. Pat. Nos. 5,143,854; 5,202,231; 5,242,974; 5,288,644; 5,324,633; 5,384,261; 5,405,783; 5,412,087; 5,424,186; 5,429,807; 5,432,049; 5,436,327; 5,445,934; 5,468,613; 5,470,710; 5,472,672; 5,492,806; 5,525,464; 5,503,980; 5,510,270; 5,525,464; 5,527,681; 5,529,756; 5,532,128; 5,545,531; 5,547,839; 5,554,501; 5,556,752; 5,561,071; 5,571,639; 5,580,726; 5,580,732; 5,593,839; 5,599,695; 5,599,672; 5,610;287; 5,624,711; 5,631,134; 5,639,603; 5,654,413; 5,658,734; 5,661,028; 5,665,547; 5,667,972; 5,695,940; 5,700,637; 5,744,305; 5,800,992; 5,807,522; 5,830,645; 5,837,196; 5,871,928; 5,847,219; 5,876,932; 5,919,626; 6,004,755; 6,087,102; 6,368,799; 6,383,749; 6,617,112; 6,638,717; 6,720,138, as well as WO 93/17126; WO 95/11995; WO 95/21265; WO 95/21944; WO 95/35505; WO 96/31622; WO 97/10365; WO 97/27317; WO 99/35505; WO 09923256; WO 09936760; W00138580; WO 0168255; WO 03020898; WO 03040410; WO 03053586; WO 03087297; WO 03091426; W003100012; WO 04020085; WO 04027093; EP 373 203; EP 785 280; EP 799 897 and UK 8 803 000; the disclosures of which are all herein incorporated by reference.

It is contemplated that the arrays can be high density arrays, such that they contain 100 or more different probes. It is contemplated that they may contain 1000, 16,000, 65,000, 250,000 or 1,000,000 or more different probes. The probes can be directed to targets in one or more different organisms. The oligonucleotide probes range from 5 to 50, 5 to 45, 10 to 40, or 15 to 40 nucleotides in length in some embodiments. In certain embodiments, the oligonucleotide probes are 20 to 25 nucleotides in length.

The location and sequence of each different probe sequence in the array are generally known. Moreover, the large number of different probes can occupy a relatively small area providing a high density array having a probe density of generally greater than about 60, 100, 600, 1000, 5,000, 10,000, 40,000, 100,000, or 400,000 different oligonucleotide probes per cm^2. The surface area of the array can be about or less than about 1, 1.6, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cm^2.

Moreover, a person of ordinary skill in the art could readily analyze data generated using an array. Such protocols include information found in WO 9743450; WO 03023058; WO 03022421; WO 03029485; WO 03067217; WO 03066906; WO 03076928; WO 03093810; WO 03100448A1, all of which are specifically incorporated by reference.

In one embodiment, nuclease protection assays are used to quantify RNAs derived from the breast cancer samples. There are many different versions of nuclease protection assays known to those practiced in the art. The common characteristic that these nuclease protection assays have is that they involve hybridization of an antisense nucleic acid with the RNA to be quantified. The resulting hybrid double-stranded molecule is then digested with a nuclease that digests single-stranded nucleic acids more efficiently than double-stranded molecules. The amount of antisense nucleic acid that survives digestion is a measure of the amount of the target RNA species to be quantified. An example of a nuclease protection assay that is commercially available is the RNase protection assay manufactured by Ambion, Inc. (Austin, Tex.).

B. Proteins and Polypeptides

In other embodiments, the differential expression patterns of breast cancer biomarkers can be determined by measuring the levels of polypeptides encoded by these genes in breast cancer cells. Methods suitable for this purpose include, but are not limited to, immunoassays such as ELISA, RIA, FACS, dot blot, Western Blot, immunohistochemistry, and antibody-based radioimaging. Protocols for carrying out these immunoassays are well known in the art. Other methods such as 2-dimensional SDS-polyacrylamide gel electrophoresis can also be used. These procedures may be used to recognize any of the polypeptides encoded by the breast cancer biomarker genes described herein.

One example of a method suitable for detecting the levels of target proteins in peripheral blood samples is ELISA. In an exemplifying ELISA, antibodies capable of binding to the target proteins encoded by one or more breast cancer biomarker genes are immobilized onto a selected surface exhibiting protein affinity, such as wells in a polystyrene or polyvinylchloride microtiter plate. Then, breast cancer cell samples to be tested are added to the wells. After binding and washing to remove non-specifically bound immunocomplexes, the bound antigen(s) can be detected. Detection can be achieved by the addition of a second antibody which is specific for the target proteins and is linked to a detectable

label. Detection may also be achieved by the addition of a second antibody, followed by the addition of a third antibody that has binding affinity for the second antibody, with the third antibody being linked to a detectable label. Before being added to the microtiter plate, cells in the peripheral blood samples can be lysed using various methods known in the art. Proper extraction procedures can be used to separate the target proteins from potentially interfering substances.

In another ELISA embodiment, the breast cancer cell samples containing the target proteins are immobilized onto the well surface and then contacted with the antibodies of the invention. After binding and washing to remove non-specifically bound immunocomplexes, the bound antigen is detected. Where the initial antibodies are linked to a detectable label, the immunocomplexes can be detected directly. The immunocomplexes can also be detected using a second antibody that has binding affinity for the first antibody, with the second antibody being linked to a detectable label.

Another typical ELISA involves the use of antibody competition in the detection. In this ELISA, the target proteins are immobilized on the well surface. The labeled antibodies are added to the well, allowed to bind to the target proteins, and detected by means of their labels. The amount of the target proteins in an unknown sample is then determined by mixing the sample with the labeled antibodies before or during incubation with coated wells. The presence of the target proteins in the unknown sample acts to reduce the amount of antibody available for binding to the well and thus reduces the ultimate signal.

Different ELISA formats can have certain features in common, such as coating, incubating or binding, washing to remove non-specifically bound species, and detecting the bound immunocomplexes. For instance, in coating a plate with either antigen or antibody, the wells of the plate can be incubated with a solution of the antigen or antibody, either overnight or for a specified period of hours. The wells of the plate are then washed to remove incompletely adsorbed material. Any remaining available surfaces of the wells are then "coated" with a nonspecific protein that is antigenically neutral with regard to the test samples. Examples of these nonspecific proteins include bovine serum albumin (BSA), casein and solutions of milk powder. The coating allows for blocking of nonspecific adsorption sites on the immobilizing surface and thus reduces the background caused by nonspecific binding of antisera onto the surface.

In ELISAs, a secondary or tertiary detection means can also be used. After binding of a protein or antibody to the well, coating with a non-reactive material to reduce background, and washing to remove unbound material, the immobilizing surface is contacted with the control and/or clinical or biological sample to be tested under conditions effective to allow immunocomplex (antigen/antibody) formation. These conditions may include, for example, diluting the antigens and antibodies with solutions such as BSA, bovine gamma globulin (BGG) and phosphate buffered saline (PBS)/Tween and incubating the antibodies and antigens at room temperature for about 1 to 4 hours or at 49° C. overnight. Detection of the immunocomplex then requires a labeled secondary binding ligand or antibody, or a secondary binding ligand or antibody in conjunction with a labeled tertiary antibody or third binding ligand.

After all of the incubation steps in an ELISA, the contacted surface can be washed so as to remove non-complexed material. For instance, the surface may be washed with a solution such as PBS/Tween, or borate buffer. Following the formation of specific immunocomplexes between the test sample and the originally bound material, and subsequent washing, the occurrence of the amount of immunocomplexes can be determined.

To provide a detecting means, the second or third antibody can have an associated label to allow detection. In one embodiment, the label is an enzyme that generates color development upon incubating with an appropriate chromogenic substrate. Thus, for example, one may contact and incubate the first or second immunocomplex with a urease, glucose oxidase, alkaline phosphatase or hydrogen peroxidase-conjugated antibody for a period of time and under conditions that favor the development of further immunocomplex formation (e.g., incubation for 2 hours at room temperature in a PBS-containing solution such as PBS-Tween).

After incubation with the labeled antibody, and subsequent to washing to remove unbound material, the amount of label is quantified, e.g., by incubation with a chromogenic substrate such as urea and bromocresol purple or 2,2'-azido-di-(3-ethyl)-benzhiazoline-6-sulfonic acid (ABTS) and hydrogen peroxide, in the case of peroxidase as the enzyme label. Quantitation can be achieved by measuring the degree of color generation, e.g., using a spectrophotometer.

Another suitable method is RIA (radioimmunoassay). An example of RIA is based on the competition between radiolabeled-polypeptides and unlabeled polypeptides for binding to a limited quantity of antibodies. Suitable radiolabels include, but are not limited to, I^{125}. In one embodiment, a fixed concentration of I^{125}-labeled polypeptide is incubated with a series of dilution of an antibody specific to the polypeptide. When the unlabeled polypeptide is added to the system, the amount of the I^{125}-polypeptide that binds to the antibody is decreased. A standard curve can therefore be constructed to represent the amount of antibody-bound I^{125}-polypeptide as a function of the concentration of the unlabeled polypeptide. From this standard curve, the concentration of the polypeptide in unknown samples can be determined. Various protocols for conducting RIA to measure the levels of polypeptides in breast cancer cell samples are well known in the art.

Suitable antibodies for this invention include, but are not limited to, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, single chain antibodies, Fab fragments, and fragments produced by a Fab expression library.

Antibodies can be labeled with one or more detectable moieties to allow for detection of antibody-antigen complexes. The detectable moieties can include compositions detectable by spectroscopic, enzymatic, photochemical, biochemical, bioelectronic, immunochemical, electrical, optical or chemical means. The detectable moieties include, but are not limited to, radioisotopes, chemiluminescent compounds, labeled binding proteins, heavy metal atoms, spectroscopic markers such as fluorescent markers and dyes, magnetic labels, linked enzymes, mass spectrometry tags, spin labels, electron transfer donors and acceptors, and the like.

Protein array technology is discussed in detail in Pandey and Mann (2000) and MacBeath and Schreiber (2000), each of which is herein specifically incorporated by reference. These arrays typically contain thousands of different proteins or antibodies spotted onto glass slides or immobilized in tiny wells and allow one to examine the biochemical activities and binding profiles of a large number of proteins at once. To examine protein interactions with such an array, a labeled protein is incubated with each of the target proteins immobilized on the slide, and then one determines which of the many proteins the labeled molecule binds. In certain

embodiments such technology can be used to quantitate a number of proteins in a sample, such as a breast cancer biomarker proteins.

The basic construction of protein chips has some similarities to DNA chips, such as the use of a glass or plastic surface dotted with an array of molecules. These molecules can be DNA or antibodies that are designed to capture proteins. Defined quantities of proteins are immobilized on each spot, while retaining some activity of the protein. With fluorescent markers or other methods of detection revealing the spots that have captured these proteins, protein microarrays are being used as powerful tools in high-throughput proteomics and drug discovery.

The earliest and best-known protein chip is the ProteinChip by Ciphergen Biosystems Inc. (Fremont, Calif.). The ProteinChip is based on the surface-enhanced laser desorption and ionization (SELDI) process. Known proteins are analyzed using functional assays that are on the chip. For example, chip surfaces can contain enzymes, receptor proteins, or antibodies that enable researchers to conduct protein-protein interaction studies, ligand binding studies, or immunoassays. With state-of-the-art ion optic and laser optic technologies, the ProteinChip system detects proteins ranging from small peptides of less than 1000 Da up to proteins of 300 kDa and calculates the mass based on time-of-flight (TOF).

The ProteinChip biomarker system is the first protein biochip-based system that enables biomarker pattern recognition analysis to be done. This system allows researchers to address important clinical questions by investigating the proteome from a range of crude clinical samples (i.e., laser capture microdissected cells, biopsies, tissue, urine, and serum). The system also utilizes biomarker pattern software that automates pattern recognition-based statistical analysis methods to correlate protein expression patterns from clinical samples with disease phenotypes.

In other aspects, the levels of polypeptides in samples can be determined by detecting the biological activities associated with the polypeptides. If a biological function/activity of a polypeptide is known, suitable in vitro bioassays can be designed to evaluate the biological function/activity, thereby determining the amount of the polypeptide in the sample.

III. Breast Cancer Therapy

Certain embodiments are directed to methods of treating breast cancer based on GR status of the breast cancer tissue. In some embodiments, the hormone receptor status is determined based on the expression of a hormone receptor such as the estrogen receptor (ER) in combination with the glucocorticoid receptor (GR).

In certain aspects, the hormone receptor status is high for GR and may also be low for one or more other hormone receptors such as the estrogen receptor. An individual having an elevated GR and low ER is likely to have a poor prognosis. In the event of a poor prognosis the physician may pursue a more aggressive therapy for those patients. In some embodiments, the method comprises identifying a breast cancer patient based on a hormone receptor status of patients having tumor tissue with elevated levels of GR expression.

In certain aspects, there may be provided methods for treating a subject determined to have cancer and with a predetermined expression profile of one or more biomarkers disclosed herein.

In a further aspect, biomarkers and related systems that can establish a prognosis of cancer patients in this invention can be used to identify patients who may get benefit of conventional single or combined modality therapy. In the same way, the invention can identify those patients who do not get much benefit from such conventional single or combined modality therapy and can offer them alternative treatment(s).

In certain aspects of the present invention, conventional cancer therapy may be applied to a subject wherein the subject is identified or reported as having a good prognosis based on the assessment of the biomarkers as disclosed. On the other hand, at least an alternative cancer therapy may be prescribed, as used alone or in combination with conventional cancer therapy, if a poor prognosis is determined by the disclosed methods, systems, or kits.

Embodiments concern a glucocorticoid receptor antagonist. In some embodiments, the glucocorticoid receptor antagonist is a selective glucocorticoid receptor antagonist, as set forth in Clark, 2008, which is hereby incorporated by reference. In other embodiments, the glucocorticoid receptor antagonist is a non-selective glucocorticoid receptor antagonist, such as mifepristone. In certain embodiments, the glucocorticoid receptor antagonist is steroidal. In other embodiments, the glucocorticoid receptor antagonist is non-steroidal. A glucocorticoid receptor antagonist includes those in the following classes of chemical compounds: octahydrophenanthrenes, spirocyclic dihydropyridines, triphenylmethanes and diaryl ethers, chromenes, dibenzyl anilines, dihydroisoquinolines, pyrimidinediones, azadecalins, and aryl pyrazolo azadecalins, and which are described in more detail in Clark, 2008, which is hereby incorporated by reference. Some embodiments of steroidal antagonists from Clark, 2008 are: RU-486, RU-43044, 11-monoaryl and 11,21 bisaryl steroids (including 11β-substituted steroids), 10β-substituted steroids, 11β-aryl conjugates of mifepristone, and phosphorous-containing mifepristone analogs. Further embodiments of nonsteroidal antagonists from Clark, 2008 are: octahydrophenanthrenes, spirocyclic dihydropyridines, triphenylmethanes and diaryl ethers, chromenes, dibenzyl anilines, dihyrdroquinolines, pyrimidinediones, azadecalins, aryl pyrazolo azadecalins (including 8a-benzyl isoquinolones, N-substituted derivatives, bridgehead alcohol and ethers, bridgehead amines). Additional specific examples include, but are not limited to the following specific antagonists: beclometasone, betamethasone, budesonide, ciclesonide, flunisolide, fluticasone, mifepristone, mometasone, and triamcinolone. Other examples include those described and/or depicted in U.S. Patent Application Publication 2010/0135956, which is hereby incorporated by reference. Even further examples include ORG-34517 (Merck), RU-43044, dexamethasone mesylate (Dex-Mes), dexamethasone oxetanone (Dex-Ox), deoxycorticosterone (DOC) (Peeters et al., 2008, which is hereby incorporated by reference in its entirety and Cho et al. 2005, which is hereby incorporated by reference in its entirety). In additional embodiments the glucocorticoid receptor antagonist may be CORT 0113083 or CORT 00112716, which are described in Belanoff et al. (2011), which is hereby incorporated by reference. It is specifically contemplated that one or more of the antagonists discussed herein or in the incorporated references may be excluded in embodiments of the invention. It is also contemplated that in some embodiments, more than one glucocorticoid receptor antagonist is employed, while in other embodiments, only one is employed as part of the therapeutic method (though it may be administered multiple times). It is contemplated that the second one may be administered concurrently with the first one or they may be administered at different times.

Conventional cancer therapies include one or more selected from the group of chemical or radiation based

treatments and surgery. Chemotherapies include, for example, cisplatin (CDDP), carboplatin, procarbazine, mechlorethamine, cyclophosphamide, camptothecin, ifosfamide, melphalan, chlorambucil, busulfan, nitrosurea, dactinomycin, daunorubicin, doxorubicin, bleomycin, plicomycin, mitomycin, etoposide (VP16), tamoxifen, raloxifene, estrogen receptor binding agents, taxol, gemcitabine, navelbine, farnesyl-protein transferase inhibitors, transplatinum, 5-fluorouracil, vincristin, vinblastin and methotrexate, or any analog or derivative variant of the foregoing.

Suitable therapeutic agents include, for example, vinca alkaloids, agents that disrupt microtubule formation (such as colchicines and its derivatives), anti-angiogenic agents, therapeutic antibodies, EGFR targeting agents, tyrosine kinase targeting agent (such as tyrosine kinase inhibitors), serine kinase targeting agents, transitional metal complexes, proteasome inhibitors, antimetabolites (such as nucleoside analogs), alkylating agents, platinum-based agents, anthracycline antibiotics, topoisomerase inhibitors, macrolides, therapeutic antibodies, retinoids (such as all-trans retinoic acids or a derivatives thereof); geldanamycin or a derivative thereof (such as 17-AAG), and other standard chemotherapeutic agents well recognized in the art.

Certain chemotherapeutics are well known for use against breast cancer. These breast cancer chemotherapeutics are capecitabine, carboplatin, cyclophosphamide (Cytoxan), daunorubicin, docetaxel (Taxotere), doxorubicin (Adriamycin), epirubicin (Ellence), fluorouracil (also called 5-fluorouracil or 5-FU), gemcitabine, eribulin, ixabepilone, methotrexate, mitomycin C, mitoxantrone, paclitaxel (Taxol), thiotepa, vincristine, vinorelbine.

In some embodiments, the chemotherapeutic agent is any of (and in some embodiments selected from the group consisting of) adriamycin, colchicine, cyclophosphamide, actinomycin, bleomycin, daunorubicin, doxorubicin, epirubicin, mitomycin, methotrexate, mitoxantrone, fluorouracil, carboplatin, carmustine (BCNU), methyl-CCNU, cisplatin, etoposide, interferons, camptothecin and derivatives thereof, phenesterine, taxanes and derivatives thereof (e.g., paclitaxel and derivatives thereof, taxotere and derivatives thereof, and the like), topetecan, vinblastine, vincristine, tamoxifen, piposulfan, nab-5404, nab-5800, nab-5801, Irinotecan, HKP, Ortataxel, gemcitabine, Herceptin®, vinorelbine, Doxil®, capecitabine, Gleevec®, Alimta®, Avastin®, Velcade®, Tarceva®, Neulasta®, Lapatinib, STI-571, ZD1839, Iressa® (gefitinib), SH268, genistein, CEP2563, SU6668, SU11248, EMD121974, and Sorafenib.

In some embodiments, the chemotherapeutic agent is a composition comprising nanoparticles comprising a thiocolchicine derivative and a carrier protein (such as albumin).

In further embodiments a combination of chemotherapeutic agents is administered to breast cancer cells. The chemotherapeutic agents may be administered serially (within minutes, hours, or days of each other) or in parallel; they also may be administered to the patient in a pre-mixed single composition. The composition may or may not contain a glucocorticoid receptor antagonist. Combinations of breast cancer therapeutics include, but are not limited to the following: AT (Adriamycin and Taxotere), AC±T: (Adriamycin and Cytoxan, with or without Taxol or Taxotere), CMF (Cytoxan, methotrexate, and fluorouracil), CEF (Cytoxan, Ellence, and fluorouracil), FAC (fluorouracil, Adriamycin, and Cytoxan), CAF (Cytoxan, Adriamycin, and fluorouracil) (the FAC and CAF regimens use the same medicines but use different doses and frequencies), TAC (Taxotere, Adriamycin, and Cytoxan), and GET (Gemzar, Ellence, and Taxol). In some embodiments trastuzumab (Herceptin®) is administered to a breast cancer patient with a glucocorticoid receptor antagonist, which may be with or without a chemotherapeutic or a combination of chemotherapeutics.

Various combinations with a glucocorticoid receptor antagonist and an anticancer agent or compound (or a combination of such agents and/or compounds) may be employed, for example glucocorticoid receptor antagonist is "A" and the anticancer agent or compound (or a combination of such agents and/or compounds) given as part of an anticancer therapy regime, is "B":

A/B/A	B/A/B	B/B/A	A/A/B	A/B/B	B/A/A	A/B/B/B	B/A/B/B		
B/B/B/A	B/B/A/B	A/A/B/B	A/B/A/B	A/B/B/A	B/B/A/A				
B/A/B/A	B/A/A/B	A/A/A/B	B/A/A/A	A/B/A/A	A/A/B/A				

Administration of the therapeutic compounds or agents to a patient will follow general protocols for the administration of such compounds, taking into account the toxicity, if any, of the therapy. It is expected that the treatment cycles would be repeated as necessary. It also is contemplated that various standard therapies, as well as surgical intervention, may be applied in combination with the described therapy.

The term "a serine/threonine kinase inhibitor", as used herein, relates to a compound which inhibits serine/threonine kinases. An example of a target of a serine/threonine kinase inhibitor includes, but is not limited to, dsRNA-dependent protein kinase (PKR). Examples of indirect targets of a serine/threonine kinase inhibitor include, but are not limited to, MCP-1, NF-kappaB, eIF2alpha, COX2, RANTES, IL8,CYP2A5, IGF-1, CYP2B1, CYP2B2, CYP2H1, ALAS-1, HIF-1, erythropoietin and/or CYP1A1. An example of a serine/theronin kinase inhibitor includes, but is not limited to, Sorafenib and 2-aminopurine, also known as 1H-purin-2-amine(9CI). Sorafenib is marketed as NEXAVAR.

The term "an angiogenesis inhibitor", as used herein, relates to a compound which targets, decreases or inhibits the production of new blood vessels. Targets of an angiogenesis inhibitor include, but are not limited to, methionine aminopeptidase-2 (MetAP-2), macrophage inflammatory protein-1 (MIP-1a), CCL5, TGF-.beta., lipoxygenase, cyclooxygenase, and topoisomerase. Indirect targets of an angiogenesis inhibitor include, but are not limited to, p21, p53, CDK2 and collagen synthesis. Examples of an angiogenesis inhibitor include, but are not limited to, Fumagillin, which is known as 2,4,6,8-decatetraenedioic acid, mono[3R, 4S,5S,6R)-5-methoxy-4-[(2R,3R)-2-methyl-3-(3-methyl-2-butenyl)oxi-ranyl]-1-oxaspiro[2.5]oct-6-yl]ester, (2E,4E, 6E,8E)-(9CI); Shikonin, which is also known as 1,4-naphthalenedione, 5,8-dihydroxy-2-[(1R)-1-hydroxy-4-methyl-3-pentenyl]-(9CI); Tranilast, which is also known as benzoic acid, 2-[[3-(3,4-dimethoxyphenyl)-1-oxo-2-propenyl]amino]-(9CI); ursolic acid; suramin; thalidomide and lenalidomide, and marketed as REVLIMID.

Radiation therapy that cause DNA damage and have been used extensively include what are commonly known as γ-rays, X-rays, and/or the directed delivery of radioisotopes to tumor cells. Other forms of DNA damaging factors are also contemplated such as microwaves and UV-irradiation. It is most likely that all of these factors effect a broad range of damage on DNA, on the precursors of DNA, on the replication and repair of DNA, and on the assembly and maintenance of chromosomes. Dosage ranges for X-rays range from daily doses of 50 to 200 roentgens for prolonged periods of time (3 to 4 wk), to single doses of 2000 to 6000

roentgens. Dosage ranges for radioisotopes vary widely, and depend on the half-life of the isotope, the strength and type of radiation emitted, and the uptake by the neoplastic cells.

The terms "contacted" and "exposed," when applied to a cell, are used herein to describe the process by which a therapeutic construct and a chemotherapeutic or radiotherapeutic agent are delivered to a target cell or are placed in direct juxtaposition with the target cell. To achieve cell killing or stasis, both agents are delivered to a cell in a combined amount effective to kill the cell or prevent it from dividing.

Approximately 60% of persons with cancer will undergo surgery of some type, which includes preventative, diagnostic or staging, curative and palliative surgery. Curative surgery is a cancer treatment that may be used in conjunction with other therapies, such as the treatment of the present invention, chemotherapy, radiotherapy, hormonal therapy, gene therapy, immunotherapy and/or alternative therapies.

Curative surgery includes resection in which all or part of cancerous tissue is physically removed, excised, and/or destroyed. Tumor resection refers to physical removal of at least part of a tumor. In addition to tumor resection, treatment by surgery includes laser surgery, cryosurgery, electrosurgery, and microscopically controlled surgery (Mohs' surgery). It is further contemplated that the present invention may be used in conjunction with removal of superficial cancers, precancers, or incidental amounts of normal tissue.

Laser therapy is the use of high-intensity light to destroy tumor cells. Laser therapy affects the cells only in the treated area. Laser therapy may be used to destroy cancerous tissue and relieve a blockage in the esophagus when the cancer cannot be removed by surgery. The relief of a blockage can help to reduce symptoms, especially swallowing problems.

Photodynamic therapy (PDT), a type of laser therapy, involves the use of drugs that are absorbed by cancer cells; when exposed to a special light, the drugs become active and destroy the cancer cells. PDT may be used to relieve symptoms of esophageal cancer such as difficulty swallowing.

Upon excision of part of all of cancerous cells, tissue, or tumor, a cavity may be formed in the body. Treatment may be accomplished by perfusion, direct injection or local application of the area with an additional anti-cancer therapy. Such treatment may be repeated, for example, every 1, 2, 3, 4, 5, 6, or 7 days, or every 1, 2, 3, 4, and 5 weeks or every 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months. These treatments may be of varying dosages as well. A patient may be administered a single compound or a combination of compounds described herein in an amount that is, is at least, or is at most 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100 mg/kg (or any range derivable therein). A patient may be administered a single compound or a combination of compounds described herein in an amount that is, is at least, or is at most 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 310, 320, 330, 340, 350, 360, 370, 380, 390, 400, 410, 420, 430, 440, 441, 450, 460, 470, 480, 490, 500 mg/kg/day (or any range derivable therein).

Alternative cancer therapy include any cancer therapy other than surgery, chemotherapy and radiation therapy in the present invention, such as immunotherapy, gene therapy, hormonal therapy or a combination thereof. Subjects identified with poor prognosis using the present methods may not have favorable response to conventional treatment(s) alone and may be prescribed or administered one or more alternative cancer therapy per se or in combination with one or more conventional treatments.

For example, the alternative cancer therapy may be a targeted therapy. The targeted therapy may be an anti-EGFR treatment. In one embodiment of the method of the invention, the anti-EGFR agent used is a tyrosine kinase inhibitor. Examples of suitable tyrosine kinase inhibitors are the quinazoline derivatives described in WO 96/33980, in particular gefitinib (Iressa). Other examples include quinazoline derivatives described in WO 96/30347, in particular erlotinib (Tarceva), dual EGFR/HER2 tyrosine kinase inhibitors, such as lapatinib, or pan-Erb inhibitors. In a preferred embodiment of the method or use of the invention, the anti-EGFR agent is an antibody capable of binding to EGFR, i.e. an anti-EGFR antibody.

In a further embodiment, the anti-EGFR antibody is an intact antibody, i.e. a full-length antibody rather than a fragment. An anti-EGFR antibody used in the method of the present invention may have any suitable affinity and/or avidity for one or more epitopes contained at least partially in EGFR. Preferably, the antibody used binds to human EGFR with an equilibrium dissociation constant (K_D) of 10^{-8}M or less, more preferably 10^{-10} M or less.

Particularly antibodies for use in the present invention include zalutumumab (2F8,), cetuximab (Erbitux), nimotuzumab (h-R3), panitumumab (ABX-EGF), and matuzumab (EMD72000), or a variant antibody of any of these, or an antibody which is able to compete with any of these, such as an antibody recognizing the same epitope as any of these. Competition may be determined by any suitable technique. In one embodiment, competition is determined by an ELISA assay. Often competition is marked by a significantly greater relative inhibition than 5% as determined by ELISA analysis.

Immunotherapeutics, generally, rely on the use of immune effector cells and molecules to target and destroy cancer cells. The immune effector may be, for example, an antibody specific for some marker on the surface of a tumor cell. The antibody alone may serve as an effector of therapy or it may recruit other cells to actually effect cell killing. The antibody also may be conjugated to a drug or toxin (chemotherapeutic, radionuclide, ricin A chain, cholera toxin, pertussis toxin, etc.) and serve merely as a targeting agent. Alternatively, the effector may be a lymphocyte carrying a surface molecule that interacts, either directly or indirectly, with a tumor cell target. Various effector cells include cytotoxic T cells and NK cells.

Gene therapy is the insertion of polynucleotides, including DNA or RNA, into an individual's cells and tissues to treat a disease. Antisense therapy is also a form of gene therapy in the present invention. A therapeutic polynucleotide may be administered before, after, or at the same time of a first cancer therapy. Delivery of a vector encoding a variety of proteins is encompassed within the invention. For example, cellular expression of the exogenous tumor sup-

pressor oncogenes would exert their function to inhibit excessive cellular proliferation, such as p53, p16 and C-CAM.

Additional agents to be used to improve the therapeutic efficacy of treatment include immunomodulatory agents, agents that affect the upregulation of cell surface receptors and GAP junctions, cytostatic and differentiation agents, inhibitors of cell adhesion, or agents that increase the sensitivity of the hyperproliferative cells to apoptotic inducers. Immunomodulatory agents include tumor necrosis factor; interferon alpha, beta, and gamma; IL-2 and other cytokines; F42K and other cytokine analogs; or MIP-1, MIP-1beta, MCP-1, RANTES, and other chemokines. It is further contemplated that the upregulation of cell surface receptors or their ligands such as Fas/Fas ligand, DR4 or DR5/TRAIL would potentiate the apoptotic inducing abilities of the present invention by establishment of an autocrine or paracrine effect on hyperproliferative cells. Increases intercellular signaling by elevating the number of GAP junctions would increase the anti-hyperproliferative effects on the neighboring hyperproliferative cell population. In other embodiments, cytostatic or differentiation agents can be used in combination with the present invention to improve the anti-hyperproliferative efficacy of the treatments. Inhibitors of cell adhesion are contemplated to improve the efficacy of the present invention. Examples of cell adhesion inhibitors are focal adhesion kinase (FAKs) inhibitors and Lovastatin. It is further contemplated that other agents that increase the sensitivity of a hyperproliferative cell to apoptosis, such as the antibody c225, could be used in combination with the present invention to improve the treatment efficacy.

Hormonal therapy may also be used in the present invention or in combination with any other cancer therapy previously described. The use of hormones may be employed in the treatment of certain cancers such as breast, prostate, ovarian, or cervical cancer to lower the level or block the effects of certain hormones such as testosterone or estrogen. This treatment is often used in combination with at least one other cancer therapy as a treatment option or to reduce the risk of metastases.

II. Kits

Certain aspects of the present invention also encompass kits for performing the diagnostic and prognostic methods of the invention. Such kits can be prepared from readily available materials and reagents. For example, such kits can comprise any one or more of the following materials: enzymes, reaction tubes, buffers, detergent, primers, probes, antibodies. In a preferred embodiment, these kits allow a practitioner to obtain samples of neoplastic cells in blood, tears, semen, saliva, urine, tissue, serum, stool, sputum, cerebrospinal fluid and supernatant from cell lysate. In another preferred embodiment these kits include the needed apparatus for performing RNA extraction, RT-PCR, and gel electrophoresis. Instructions for performing the assays can also be included in the kits.

In a particular aspect, these kits may comprise a plurality of agents for assessing the differential expression of a plurality of biomarkers, for example, GR and/or ER, wherein the kit is housed in a container. The kits may further comprise instructions for using the kit for assessing expression, means for converting the expression data into expression values and/or means for analyzing the expression values to generate prognosis. The agents in the kit for measuring biomarker expression may comprise a plurality of PCR probes and/or primers for qRT-PCR and/or a plurality of antibody or fragments thereof for assessing expression of the biomarkers. In another embodiment, the agents in the kit for measuring biomarker expression may comprise an array of polynucleotides complementary to the mRNAs of the biomarkers of the invention. Possible means for converting the expression data into expression values and for analyzing the expression values to generate scores that predict survival or prognosis may be also included.

Kits may comprise a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container may hold a composition which includes a probe that is useful for prognostic or non-prognostic applications, such as described above. The label on the container may indicate that the composition is used for a specific prognostic or non-prognostic application, and may also indicate directions for either in vivo or in vitro use, such as those described above. The kit of the invention will typically comprise the container described above and one or more other containers comprising materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes, and package inserts with instructions for use.

EXAMPLES

The following examples are given for the purpose of illustrating various embodiments of the invention and are not meant to limit the present invention in any fashion. One skilled in the art will appreciate readily that the present invention is well adapted to carry out the objects and obtain the ends and advantages mentioned, as well as those objects, ends and advantages inherent herein. The present examples, along with the methods described herein are presently representative of preferred embodiments, are exemplary, and are not intended as limitations on the scope of the invention. Changes therein and other uses which are encompassed within the spirit of the invention as defined by the scope of the claims will occur to those skilled in the art.

Example 1

Tumor Biomarker Status

A. Results

The glucocorticoid receptor (GR) is highly expressed in the myoepithelium of the normal human breast and in a subset of both ERalpha-positive and negative human breast cancers. In vitro and in vivo experiments suggest that activation of the GR in ER− pre-malignant breast epithelial and cancer cells triggers cell survival pathways under stress conditions (e.g. chemotherapy) that usually induce apoptosis. The inventors examined the association between NR3C1 gene expression and GR target gene expression in human ER− breast cancers and found that ER− breast cancers with high NR3C1 expression also express GR target genes associated with EMT and anti-apoptotic signaling, and that those ER− patients with high NR3C1 gene expression have a significantly worse outcome than NR3C1-low patients. Interestingly, the high NR3C1 gene expression in the ER+ (ESR1-high) subset of patients suggests a slight better outcome, implying a crosstalk between the ER and the GR that is absent in ER− tumors.

Using a global approach of gene expression studies merged with data from GR ChIP-sequencing in ER− pre-malignant breast cells (MCF10A-Myc), the inventors have identified direct GR target genes are significantly associated with cell survival signaling pathways. Interestingly, a meta-

analysis of the high NR3C1-expressing ER− tumors reveals that many genes identified by ChIP-sequencing/gene expression analysis are indeed differentially expressed in high versus low NR3C1-primary breast cancers. These results suggest that GR expression may be a functional biomarker in ER− breast cancer.

TABLE 1

Clinical studies used for meta-analysis

GEO ID	# of pts	Reference
GSE9195	77	Loi S, et al
GSE7390	189	Desmedt C, et al
GSE6532	212	Loi S, et al
GSE2603	73	Minn A J, et al
GSE2990	183	Sotiriou C, et al
GSE2034	280	Wang Y X, et al
TOTAL	1206	

TABLE 2

Differentially expressed genes with concordant expression by all three methods (33/44 genes)

Gene expression after Dex-treatment in MCF10A-Myc	Gene expression in NR3C1 + vs. − tumors	GR-binding within distance to TSS after Dex-treatment in MCF10A-Myc	Genes
Up	Up	10 kb	DUSP1, SGK1, SMARCA2, PTGDS, MCL1
		10-100 kb	DPYSL2, STOM, LAPTM5, NNMT, SERPINF1, NRIP1, WIPF1, BIN1, IL1R1, ST3GAL5, SEMA4D, MAP3K5, SMARCA2, DPT, BIRC3, PTGDS, PHF15, MAOA, TFPI, SLC46A3, PIAS1, ACSL5, SESN1, C14orf139, LBH
Down	Down	10 kb	NONE
		10-100 kb	SFN, SPP1, ERBB2
Overlapping genes with NKI-285 gene signature			DUSP1, DPT, NNMT, SERPINF1, IL1R1, FN1, DPYSL2

B. Materials and Methods

Cell culture and glucocorticoid treatment: MCF10A-Myc cells were cultured in a 1:1 mixture of DMEM and Hams/F12 medium supplemented with 10% fetal bovine serum, hydrocortisone (0.5 μg/ml), EGF(10 ng/ml), insulin (5 ng/ml) and 100 U/ml penicillin/streptomycin were also added. The cells were then starved for three days of all growth factors and treated with dexamethasone (10-6M) and ethanol of the same volume as a control.

Microarray gene expression: MCF10A-Myc Cells: Time course (0.5 h, 2 h, 4 h and 24 h) microarray data were obtained using Affymetrix gene arrays (HG-U133A) (Wu et al., 2006). Genes that were induced or repressed ≥1.5 fold-change were considered to be regulated.

GR ChIP-Seq experiment and analysis for MCF10A-Myc Cells: Cells were collected for the ChIP assay following 1 hour of Dex (10-6M) or EtOH treatment. The ChIP assay was done basically following Millipore's ChIP Assay Kit instructions. The DNA input (1%) was also sequenced using Illumina's Solexa Sequencer. Short-tag reads (36 bp) were mapped to the Human Genome (UCSC, hg18) by using Maq aligner. GR-binding peaks were called by using MACS software. Known SGK1 and GILZ promoter GR binding-regions (GBRs) were used as positive controls to determine the FDR threshold for retrieving significant GBRs.

Human Primary Breast Cancer Analysis: 1) Data Collection: All the clinical data and raw CEL files (all Affymetrix HU-133A and HU-133+2) were obtained from GEO (see Table 1). Low quality arrays were removed by AffyPLM. Arrays were normalized by using RMA and then centered by mean within each study and pooled together. 2) Determination of ESR1 and NR3C1 positivity: Expression data of tumors with known ER IHC status were analyzed using ROC analysis. The Youden Index of the best ESR1 probe's ROC curve was used as the cut-off point to separate ESR1+ and ESR1− tumors. Due to the lack of tumors with both GR IHC and NR3C1 gene expression information, we were unable to use ROC analysis to determine the NR3C1 cutoff. Therefore, based on published and our unpublished GR IHC data, we used the percentiles of NR3C1 gene expression levels that correspond to the observed proportion of GR+ patients. 3) Clustering: Un-supervised clustering was performed by Cluster using Pearson correlation distance and complete-linkage method. Heat-maps were plotted by Treeview. 4) Statistical analysis: Relapse-free survival (RFS) Kaplan-Meier plot and log-rank test were done by using R's "survival" package. Microarray SAM analysis was performed by using R's "siggenes" package.

Tumor assessment. pAUC areas were calculated for all the probes on the chip by setting p=0.2 (meaning can separate at least 80% patients) for tumors with known ER status (n=1000). A probe was then selected that has biggest pAUC area, which is the ESR1 probe 205225_at. So, this probe is the best one that can separate ER IHC+versus −. Using the 205225_at probe, the Youden Index of its ROC curve was calculated, that is the max (sensitivity+specificity−1) as the cut-off value for ESR1+ and −. The range of ESR1 expression after normalization was [−5.223868-3.944120]. The Youden Index, i.e. the cut-off is −1.257434. In the n=1000, training set, n=773>−1.257434 (ESR1+), and n=227 <=−1.257434. (ESR1−) or i.e. 77.3% quantile

This cut-off was applied to the entire dataset, n=898 (ESR+), n=308 (ESR−). In addition to the method, the ACTUAL Log 2 value cutoff is needed for ESR1 positivity in normalized meta-dataset, as well as the range of ESR1 values encountered following batched mean normalization. If in one study, samples are obtained from different hospitals, they were normalized separately. So, to be precisely accurate, the normalization is done within the samples from the same source.

The ESR1 probe ID from Affymetrix is 205225_at.

The NR3C1 probe ID from Affymetrix is 216321_s_at

The range for NR3C1 probe (216321_s_at) is [−3.145456 to 2.158716] for the entire data set. For ESR1+, the range is [−3.009359 2.158716] and for ESR1−, the range is [−3.145456 1.917823] Thus, the cut-off for ESR1+, is 0.172189, 55.98% quantile (or about 44% NR3C1+ percentage) and the cut-off for ESR1−, is 0.47332, 82.51% quantile (or about 17.5% NR3C1+ percentage). All the cut-off are log 2 values.

The cutoffs used are the best cut-off that can separate patients with a $p<0.01$. If the p-value is loosened to 0.05, the range can be widened.

For ESR1+ patients, NR3C1+ patients can be from about 35% to 60% (about 44% is the best). For ESR1− patients, NR3C1+ patients can be from about 30% to 15% (about 17.5% is the best)

Example 2

Mifepristone Pretreatment Enhances Paclitaxel Anti-Tumor Effectiveness in Models of Human Breast Cancer

Xenografted ER−/PR−/HER2− (GR+) MDA-MB-231 human breast cancer cells (1×10^7 cells in 50 μl of PBS) were injected into the mammary fat pad of female Severe Combined Immunodeficient Mice (SCID) mice and allowed to grow until reaching approximately 100 mm^3. Mice were then injected intraperitoneally with either both vehicles, paclitaxel (10 mg/kg)+ the mifepristone vehicle, or the combination of mifepristone (15 mg/kg) administered two hours prior to paclitaxel (10 mg/kg) for five successive days. The longest (L) and shortest (S) diameters of the tumors were measured bi-weekly with electronic calipers and tumor volume was calculated using the formula for an ellipsoid sphere: volume=S2×L×0.52. Mifepristone pretreatment significantly decreased tumor volume over time (P=0.013) compared to treatment with paclitaxel alone (FIG. **8**).

Example 3

Mifepristone Pretreatment Increases Tamoxifen-Resistant MCF-7 (T-R-MCF-7), but not Parental MCF-7 Cell Susceptibility to Paclitaxel in Vitro

Parental MCF-7 (ER+/PR+/GR+) and T-R MCF-7 (ER+/PR+/GR+) cells were treated with the appropriate vehicle (ethanol for mifepristone and castor oil/saline for paclitaxel), paclitaxel alone (10^{-6} M), and paclitaxel/mifepristone (10^{-6} M). Apoptosis was measured using FITC conjugated-anti-Annexin V antibody labeling followed FACS analysis to determine the percentage of the total cell population undergoing apoptosis after 20 hours of treatment. Mean +/− SE is shown. Significantly more apoptosis (P=0.028) was observed in the T-R MCF-7 cells when treated with mifepristone/paclitaxel compared to paclitaxel alone (FIG. **9**). No difference was seen in the parental MCF-7 cells.

Sequence Listing

NR3C1 GenBank AY436590-127687 bp, incorporated herein by reference
ESR1 GenBank NG_008493-419779 bp, incorporated herein by reference
SEQ ID NO: 1 NR3C1 mRNA

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Sequence Listing

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Sequence Listing

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SEQ ID NO: 3-46 MCL1, SAP30, DUSP1, SGK1, SMARCA2, PTGDS, TNFRSF9, SFN, LAPTM5, GPSM2, SORT1, DPT, NRP1, ACSL5, BIRC3, NNMT, IGFBP6, PLXNC1, SLC46A3, C14orf139, PIAS1, IDH2, SERPINF1, ERBB2, PECAM1, LBH, ST3GAL5, IL1R1, BIN1, WIPF1, TFPI, FN1, FAM134A, NRIP1, RAC2, SPP1, PHF15, BTN3A2, SESN1, MAP3K5, DPYSL2, SEMA4D, STOM, and MAOA gene.

SEQ ID NO: 47 GR alpha.

SEQ ID NO: 48 GR beta.

SEQ ID NO: 49 NRR3C1 mRNA (complete)

REFERENCES

The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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U.S. Pat. No. 5,202,231
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U.S. Pat. No. 5,384,261
U.S. Pat. No. 5,405,783
U.S. Pat. No. 5,412,087
U.S. Pat. No. 5,424,186
U.S. Pat. No. 5,429,807
U.S. Pat. No. 5,432,049
U.S. Pat. No. 5,436,327
U.S. Pat. No. 5,445,934
U.S. Pat. No. 5,468,613
U.S. Pat. No. 5,470,710
U.S. Pat. No. 5,472,672
U.S. Pat. No. 5,492,806
U.S. Pat. No. 5,503,980
U.S. Pat. No. 5,510,270
U.S. Pat. No. 5,525,464
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U.S. Pat. No. 5,527,681
U.S. Pat. No. 5,529,756
U.S. Pat. No. 5,532,128
U.S. Pat. No. 5,545,531
U.S. Pat. No. 5,547,839
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<400> SEQUENCE: 5

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<400> SEQUENCE: 6

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<220> FEATURE:
<223> OTHER INFORMATION: SMARCA2 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 7

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<210> SEQ ID NO 13
<211> LENGTH: 7018
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<223> OTHER INFORMATION: SORT1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 13

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<400> SEQUENCE: 14

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<211> LENGTH: 5243
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
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<400> SEQUENCE: 17

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<211> LENGTH: 1579
<212> TYPE: DNA
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<220> FEATURE:
<223> OTHER INFORMATION: NNMT glucocorticoid receptor-responsive gene

<400> SEQUENCE: 18

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<400> SEQUENCE: 19

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<220> FEATURE:
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<400> SEQUENCE: 20

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: SLC46A3 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 21

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<210> SEQ ID NO 22
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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: C14orf139 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 22

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<220> FEATURE:
<223> OTHER INFORMATION: PIAS1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 23

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<223> OTHER INFORMATION: SERPINF1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 25

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<400> SEQUENCE: 26

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: PECAM1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 27

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<211> LENGTH: 4909
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: IL1R1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 30

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<210> SEQ ID NO 31
<211> LENGTH: 2210
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: BIN1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 31

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<210> SEQ ID NO 32
<211> LENGTH: 4664
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: WIPF1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 32

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<210> SEQ ID NO 33
<211> LENGTH: 1166
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: TFPI glucocorticoid receptor-responsive gene

<400> SEQUENCE: 33

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<210> SEQ ID NO 34
<211> LENGTH: 8449
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: FN1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 34

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<212> TYPE: DNA
<213> ORGANISM: Homo sapiens

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<211> LENGTH: 1641
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: SPP1 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 38

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<210> SEQ ID NO 39
<211> LENGTH: 6463
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: PHF15 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 39

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<220> FEATURE:
<223> OTHER INFORMATION: MAP3K5 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 42

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: DPYSL2 glucocorticoid receptor-responsive gene

<400> SEQUENCE: 43

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<210> SEQ ID NO 44
<211> LENGTH: 4417
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: SEMA4D glucocorticoid receptor-responsive gene

<400> SEQUENCE: 44

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<210> SEQ ID NO 45
<211> LENGTH: 3108
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: STOM glucocorticoid receptor-responsive gene

<400> SEQUENCE: 45

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<210> SEQ ID NO 46
<211> LENGTH: 4090
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: MAOA glucocorticoid receptor-responsive gene

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<400> SEQUENCE: 46

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aaaaaaaaaa 4090

<210> SEQ ID NO 47
<211> LENGTH: 6784
<212> TYPE: DNA
<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: glucocorticoid receptor (GR) alpha

<400> SEQUENCE: 47

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<213> ORGANISM: Homo sapiens
<220> FEATURE:
<223> OTHER INFORMATION: glucocorticoid receptor (GR) beta

<400> SEQUENCE: 48

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<213> ORGANISM: Homo sapiens
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What is claimed is:

1. A method of killing breast cancer cells comprising administering to a breast cancer patient an effective amount of a combination of anti-cancer compounds, wherein the breast cancer cells do not express detectable levels of estrogen receptor alpha, and wherein the anticancer compounds comprise a glucocorticoid receptor antagonist and a chemotherapeutic.

2. The method of claim **1**, wherein the breast cancer cells were previously administered a first chemotherapeutic more than two weeks prior to the combination of anti-cancer compounds.

3. The method of claim **2**, wherein the breast cancer cells that were previously administered a first chemotherapeutic are chemo-resistant.

4. The method of claim **3**, wherein the breast cancer cells are determined not to be chemo-sensitive or are determined to be chemo-resistant.

5. The method of claim **1**, wherein the breast cancer cells are glucocorticoid receptor-positive (GR+).

6. The method of claim **1**, wherein the patient is determined to have breast cancer cells that are GR+.

7. The method of claim **1**, wherein the combination of anti-cancer compounds is administered within 1 week of each anti-cancer compound.

8. The method of claim **7**, wherein the combination of anti-cancer compounds is administered within 24 hours of each anti-cancer compound.

9. The method of claim **7**, wherein the glucocorticoid receptor antagonist is administered prior to the chemotherapeutic.

10. The method of claim **9**, wherein the glucocorticoid receptor antagonist is administered up to three days prior to administering the chemotherapeutic.

11. The method of claim **7**, wherein the glucocorticoid receptor antagonist is administered after the chemotherapeutic is administered.

12. The method of claim **9**, wherein the glucocorticoid receptor antagonist is also administered after the chemotherapeutic is administered.

13. The method of claim **1**, wherein the glucocorticoid receptor antagonist is administered prior to and after administration of the chemotherapeutic.

14. The method of claim **13**, wherein the glucocorticoid receptor antagonist is administered up to three days prior to administration of the chemotherapeutic.

15. The method of claim **1**, wherein the glucocorticoid receptor antagonist has undetectable or a lower level of activity as a progesterone receptor antagonist.

16. The method of claim **15**, wherein the glucocorticoid receptor antagonist does not have detectable progesterone receptor antagonist activity.

17. The method of claim **1**, wherein the breast cancer is an unresectable breast cancer.

18. The method of claim **1**, wherein dexamethasone has not been administered to the patient within 24 hours of administration of the glucocorticoid receptor antagonist.

19. The method of claim **1**, wherein the breast cancer cells were previously administered a first apoptosis inducing agent more than two weeks prior to the glucocorticoid receptor antagonist.

20. The method of claim **19**, wherein at least one apoptosis inducing agent is radiation, a chemotherapeutic, or an immunotherapy.

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