



# Promoter Hypermethylation As a Molecular Marker for Breast Cancer

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# Cancer

- Over 1,300,000 new cases annually in USA
- Over 10 million Americans (in last 5 years)
- Estimated health care costs: \$190 billion (health expenditures, loss of productivity illness/death: ANNUALLY)
- It is the leading cause of death in US
- Over 1,500 die every day from cancer
- Lifetime risk
  - Women: 1 in 3
  - Men: 1 in 2

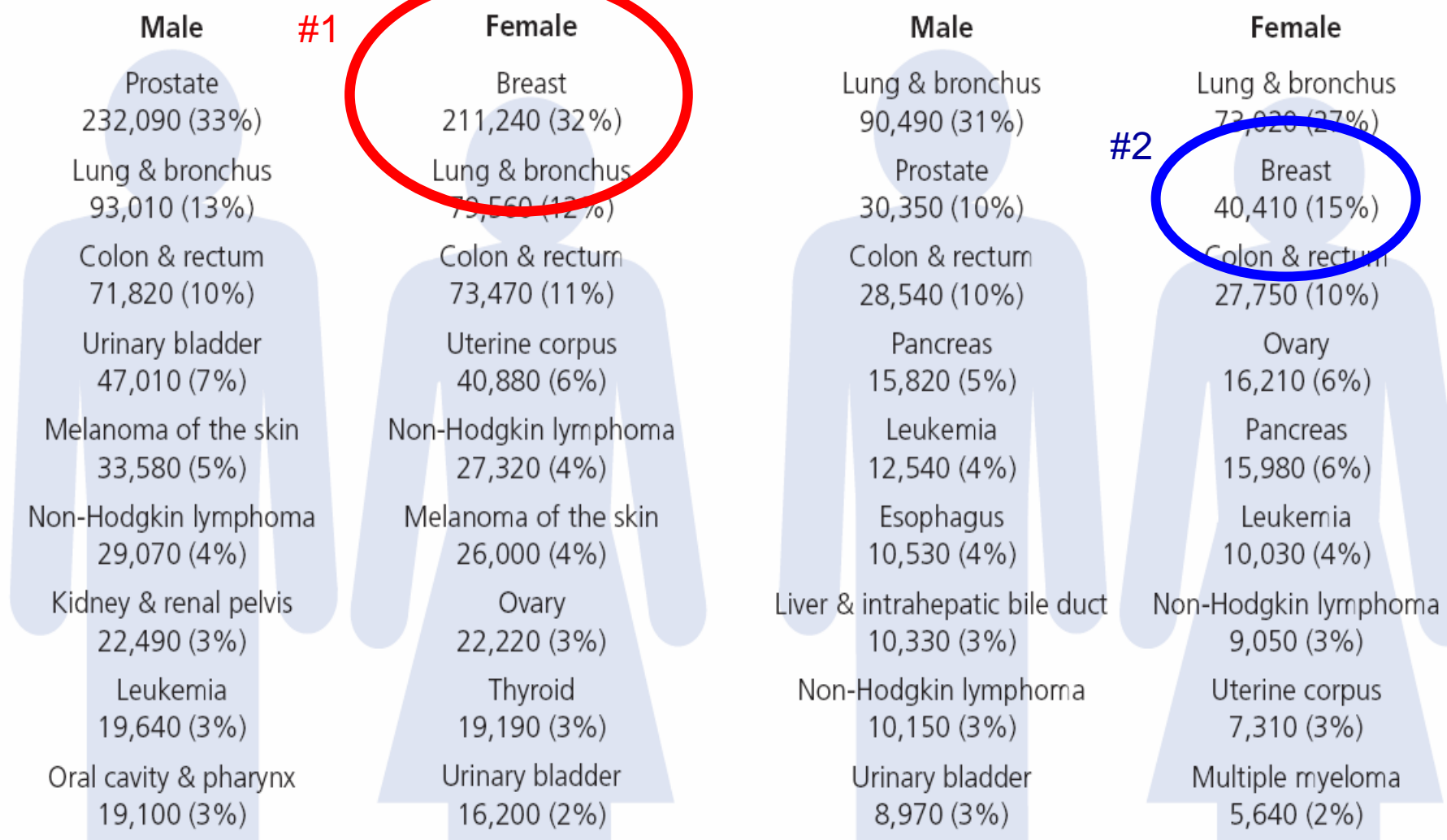
# Cancer Prevention: The reality

- 570,000 cancer deaths in 2005
- More than 175,000 cancer deaths are caused by tobacco use
- Over 190,000 deaths will be related to obesity, nutrition, and physical inactivity
- Over 1 million skin cancers diagnosed in 2005 could be prevented by protection from suns rays
- Healthcare screening for breast, prostate, cervical, colorectal and skin cancers account for half of all new cancer cases. (>680,000)
- 5 year relative survival rate for these cancers is about 85%.
- If these lesions were found at a localized stage, RSR=95%.
  
- Impact of lifestyle changes is immense

# Leading Sites of New Cancer Cases and Deaths – 2005 Estimates\*

## Estimated New Cases\*

## Estimated Deaths



# Breast Cancer

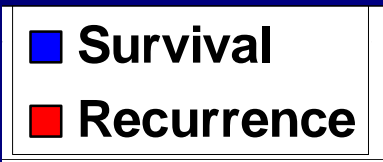
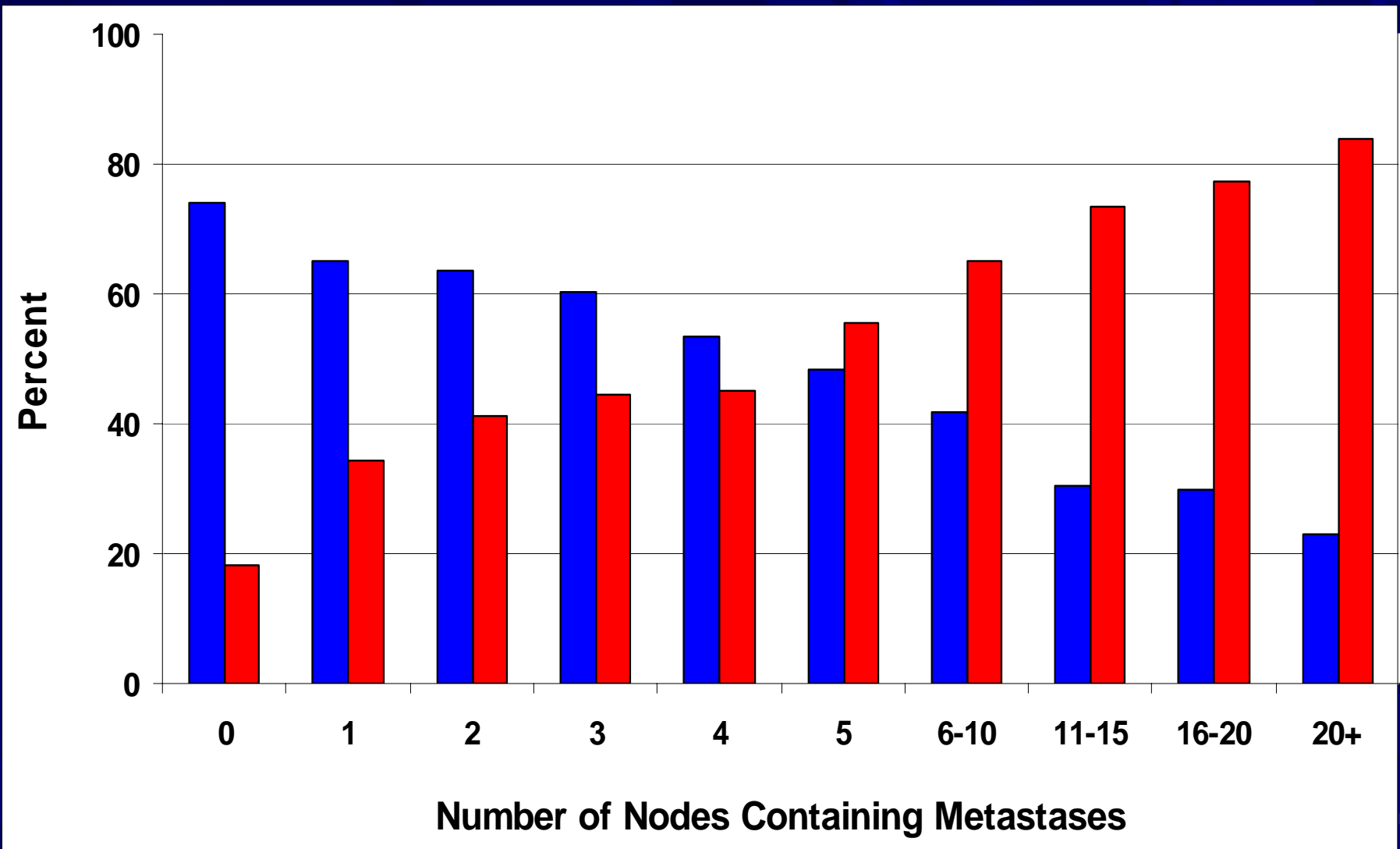
- Most frequent cancer in women
- 200,000 invasive breast cancers annually
- Additional 58,490 in situ breast cancers (85%DCIS)
- Totals 260,000 women affected in BC 2006
  
- 2nd cause of cancer death in USA (40,410)
- Incidence still increasing (gradually)
  
- Leading cause of cancer death in women from age 20-60

# Breast Cancer

- 60-70% women have localized disease (120,000 women)
- Majority cured with local resection
- Node negative pts with low risk tumor (size < 1cm, ER/PR +, well-differentiated)
- No adjuvant chemotherapy
  
- 20-30% of women with node negative disease have recurrence and die within 10 years of diagnosis (30,000 women annually)

# Sentinel node

- Lymphatic drainage of a primary tumor site goes preferentially to a specific and identifiable lymph node, the “sentinel node”
- Status of the sentinel node is predictive of the regional lymph node basin
- Lymph node status is strongly predictive of prognosis



# Hypothesis: Sentinel Node Study

Promoter hypermethylation is a molecular tool which identifies a subset of breast cancer patients with an increased risk for recurrent disease after lumpectomy.

# Design

- Prospective cohort study, started in 1998
- Newly diagnosed, clinically node negative early stage breast cancer
- Undergo lumpectomy and sentinel node biopsy as surgical staging
- Follow patients for minimum of 5 years to determine disease free survival

# Design

- Node negative patients (Over 400 patients enrolled)
  - Molecularly (MS-PCR) positive
  - Molecularly (MS-PCR) negative
- MS-PCR results will be compared with disease free survival data

**Does methylation in lymph nodes free of histological evidence of disease predict breast cancer recurrence?**

# Clinical Database:

- 112 patients: 110 F, 2 M
- Mean age 58 (34-87)
- 76 ductal, 8 lobular, 22 mixed, 6 other
- 77 ER positive, 30 ER negative, 5 unknown
- 69 PR positive, 38 PR negative, 5 unknown
- 106 no LVI, 6 yes LVI
- Ever Smokers: 38 yes, 74 no
  
- Recurrence of breast cancer
- Distant, Regional, Local recurrence
- Ave follow-up time years: mean 5.4 years
- Adjuvant Chemo: 35 yes, 77 no
- Adjuvant Endocrine tx: 64 yes, 48 no
- Adjuvant Radiation: 76 yes, 36 no
  
- Other Primaries: 7 yes, 105 no (2 endometrial, 1 neuroendocrine, 2 renal, 1 sarcoma, 1 ovarian, 1 new breast primary)

# Epigenetics

Change in gene expression which is heritable and which does not involve a change in DNA sequence (not genetic)

# DNA Methylation

- Frequent cancer finding
- Aberrant methylation of promoter region (CpG islands) is associated with loss of transcription of the involved gene
- These regions in normal tissues are unmethylated

# Methylation changes in Neoplasia

Normal



Cancer



# Which genes are methylated in breast cancer?

Gene		Freq. of M in BC
<u>AP2</u> :	Tumor suppressor	<b>70%</b> (51/73)
<u>BRCA-1</u> :	Repair DNA damage	13% (11/84)
<u>Cyclin D2</u> :	Cell cycle regulation, diffn.	46%
<u>ECAD</u> :	Tumor cell invasion	<b>26-50%</b>
<u>ER</u> :	Growth factor response	<b>50%</b>
<u>FHIT</u> :	Tumor suppressor gene	<b>48%</b> (22/46)
<u>GST-pi</u> :	Repair of DNA damage	31% (24/77)
<u>HIN-1</u> :	Tumor suppressor fxn.	<b>74%</b> (14/19)
<u>P16</u> :	Altered cell cycle control	17% (11/66)
<u>RAR-Beta</u> :	Growth factor response	25%
<u>RASSF1</u> :	Tumor suppressor	<b>49%-70%</b>

	rassf1	p16	ap2	cyclind	er	HIN 1	gstpi	FHIT	brca1	socs	rarbata	ecad	ER	gstpi	er
1	Red	Red	Red	Red	Green	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
2	Red	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
3	Red	Red	Red	Red	Red	Red	Green	Red	Red	Red	Red	Red	0	Green	Red
4	Red	Red	Red	Red	Green	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
5	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
6	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	0	Red	Red
7	Red	Red	Red	Red	Red	Red	Red	Green	Red	Red	Red	Red	0	Red	Red
8	Red	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	0	Red	Red
9	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
10	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
11	Red	Red	Green	Red	Green	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
12	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Green
13	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
14	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	0	Green	Red
15	Red	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
16	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Green	Red
17	Red	Red	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
18	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
19	Green	Red	Green	Red	Green	Red	Red	Red	Red	Red	Red	Red	0	Green	Red
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21	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Green	Red
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24	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	0	Green	Red
25	Red	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Green	Red
26	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Green	Red
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41	Red	Red	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	0	Green	Red
42	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red
43	Green	Green	Red	Red	Red	Red	Red	Red	Red	Red	Red	Red	1	Red	Red

5 panels of 3 genes were identified such that 72 of 73 women had at least 1 of 3 genes methylated.

**Panel A**

Rassf1 (32/43)

P16 (add 9)

AP2 (add 1)

**Panel B**: Cyclin d (add 1)

**Panel C**: ER (add 1)

**Panel D**: GSTp (add 1)

**Panel E**: Hin-1 (add 1)

Red=methylated  
Green=unmethylated

# Patients with node negative BC

- Over 290 samples tested w/ 8 gene panel
- 78 tumor-node pairs (many with >2nodes)
- 34 additional patients with only node tissue and no corresponding tumor tissue
- Total of over 112 patients

# Anticipated Outcomes

- MS-PCR may be a sensitive and specific molecular marker to identify women with early stage breast cancer who may be at higher risk of recurrence.
- Hopefully, this tool will be able to identify patients who warrant additional therapy and consequently improve survival.
- More importantly, will identify women who may be able to forego unnecessary therapy. (80,000 women)
- DCIS cohorts (predict recurrences). Data now completed on over 100 DCIS/IBC samples. Await clinical database correlation.
- Acquire blinded samples from separate institution and test modified gene panel, potential for 100-300 samples with (10 year) long term follow-up.

# Can we improve our understanding of tobacco's impact on breast cancer?

- Standardize and optimize patient questionnaires regarding patient smoking history for all breast cancer clinical trials.
- Improve evaluation of patients known to have significant exposure to smoking
  - Women with lung cancer...is their incidence of breast cancer different than those women in usual population?
  - Dr. Malcolm Brock data: Looked at rates of secondary cancers in 9,000 primary lung cancer patients. 7,500 had only lung cancer. 1,500 had lung plus another tumor. 615 were women and of those, 153 had breast cancer. (AACR poster 2003)

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