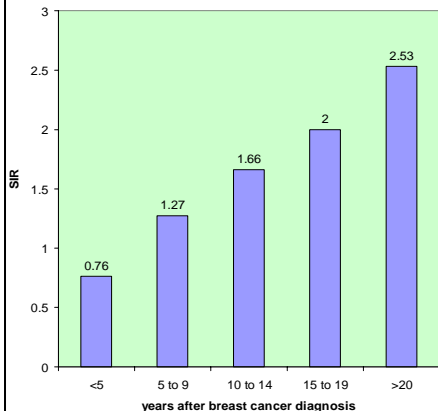


Biomarkers for susceptibility to primary lung cancer in women with breast cancer

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Fig 1. Standardized incidence ratio (SIR) for lung cancer in relation to time after initial breast cancer diagnosis. A significant risk of lung cancer was detected in a population of approximately 141,000 women with breast cancer.



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Fig. 2. Study Design

Source: Swedish Cancer Registry

Include

- 115 cases with breast and secondary lung cancer
- 115 controls with breast cancer only
- Diagnosed in Stockholm region

Exclude

- Male breast cancer
- History of any other cancer
- Lung cancer diagnosis within one year of breast cancer diagnosis
- Non-primary lung tumors
- X-ray or autopsy only diagnosis

Cases and controls are matched on age at breast cancer diagnosis and time to lung cancer diagnosis

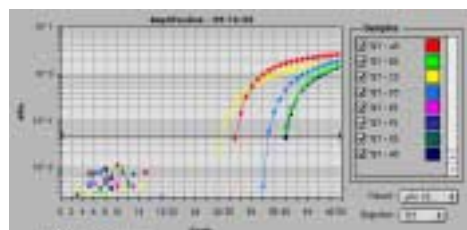
Background

- ❖ Small but real increased overall risk of lung cancer after breast cancer
- ❖ 10 years after RT for breast cancer, there is an increased risk of ipsilateral lung cancer, with more risk beyond 10 years (ref 1).
- ❖ Non-smokers with breast cancer exposed to RT have a 2-3 fold increased risk of lung cancer; smokers with no RT 13-20 fold increased risk; smokers with RT 30 fold increased risk; greatest risk for all in ipsilateral lung (ref 2).
- ❖ Preliminary data demonstrates an increased risk of lung cancer after radiotherapy for breast cancer, with an increased risk of concordant lung tumors (Fig 1).
- ❖ Study Design: 115 cases with breast cancer followed by lung cancer and matched controls with breast cancer only (Fig 2).

Aims and Hypotheses

- Determine methylation of a panel of genes.
 - ❖ 1a: the presence of hypermethylated genes in breast tumors will predict the risk of later lung cancer by case control comparison.
 - ❖ 1b: the presence of hypermethylated genes in breast tumors will be more commonly associated with the presence of hypermethylated genes in lung tumors by case only comparison.
- Determine the mutational spectra of p53 in breast and lung tumors
 - ❖ 2a: the presence of p53 mutations in breast cancer will predict the risk of subsequent lung cancer by case control comparison.
 - ❖ 2b: the presence of p53 mutations in a breast tumor will be more commonly associated with the presence of p53 mutations in the lung tumor by a case only comparison.
- A descriptive study to determine the expression of estrogen receptor (ER) alpha and beta in breast and lung tumors. The presence of ER in the lung and gender differences in lung cancer risk suggest that there may be a hormonal influence on the biology of lung cancer.
 - ❖ 4. Secondary hypotheses:
 - ❖ 4a: RT, smoking, and lung cancer risk by case control and confirm with lateral case only comparison.
 - ❖ 4b: RT, smoking, p53 mutations, and lung cancer risk by case control and case only comparison.
 - ❖ 4c: RT, smoking, the presence of hypermethylated genes, and lung cancer risk by case control and case only comparison.

Fig. 3 Real time PCR specific for methylation. NTC= no template control



A5= pos control B5= unmeth. DNA C5= pos control D5= p53 NTC F5= NTC G5= sample H5= sample

Methods

- ❖ Bisulfite modification of DNA and real time PCR to identify methylation in a panel of genes (Table 1 and Fig 3). Validation by b-actin PCR and sequencing.
- ❖ p53 Affymetrix Gene Chip assay to identify mutations. Validation by sequencing (Fig 4).
- ❖ ER alpha IHC (Fig 5). ER beta IHC will be done by researchers at KI.
- ❖ Primary lung tumor status will be verified with Thyroid Transcription Factor 1 IHC at the Karolinska Institutet (KI). (Fig 6)
- ❖ 20% repeats for quality control.

Table 1. Literature values for methylation rates of genes in study panel.

Gene	Function	% meth. in breast tumors	% meth. in lung tumors
p16	cyclin-dependent kinase inhibitor, tumor suppressor	14-18	6-41
RARβ2	Retinoic acid receptor, differentiation, tumor suppressor, growth	24-57	10-49
E-cadherin	Adhesion, invasion, metastasis	19-55	18-87
Estrogen Receptor	tumor proliferation, growth	25-49	24
O6 MGMT	DNA repair, protection from O6 guanine adducts	NA	0-39
BRCA1	DNA repair, transcription, tumor suppressor	12-57	NA

Fig. 4 p53 multiplex PCR for Affymetrix GeneChip assay on Swedish breast and lung samples from 1951-1961.

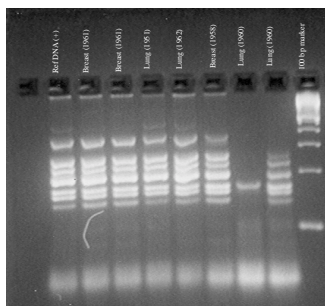
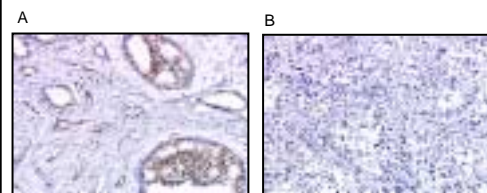


Fig 5. Preliminary immunohistochemistry (IHC) on early Swedish samples demonstrates that the tissue is suitable for staining. (A) ER alpha positive breast tissue from a tumor embedded in paraffin in 1961. (B) ER alpha negative breast tumor tissue from 1983.



Assay completion progress

Assay	#Samples Completed	Initial Results
ER Alpha IHC	300/317	breast 156/198 pos lung 11/102 pos
p53 GeneChip	168/317	breast 82/94 score of 15+ lung 42/74 score of 15+
Methylation		
O6MGMT	76/108	8/76 pos

Current Work

- ❖ Receive and process remaining 28 samples.
- ❖ Complete remaining p53 analysis and validation by sequencing.
- ❖ Complete methylation analysis and validation by sequencing.
- ❖ Complete ER alpha IHC review with pathologist.
- ❖ Analysis with researchers at the Karolinska Institutet.

Fig. 6 TTF-1 staining in (A) breast metastasis to lung and (B) lung adenocarcinoma.

