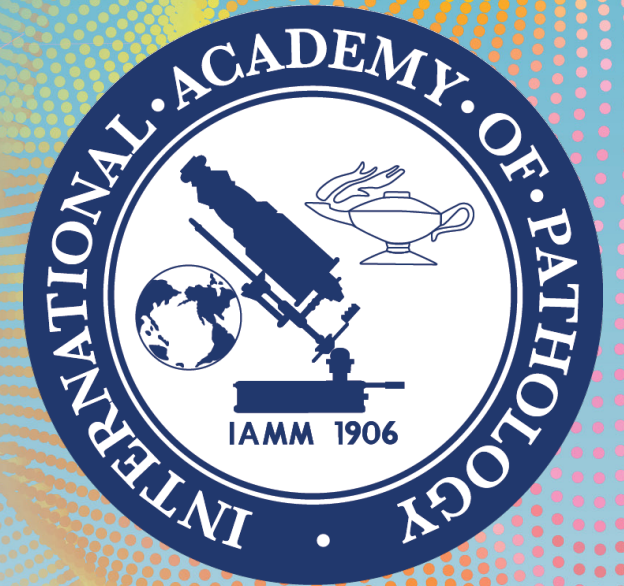


# E-cadherin, ROS1 and synthetic lethality in invasive lobular carcinoma

Anna Sokolova

Sullivan and Nicolaides Pathology, Queensland  
University of Queensland



# Disclosure of Relevant Financial Relationships

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No relevant financial relationships

# Background

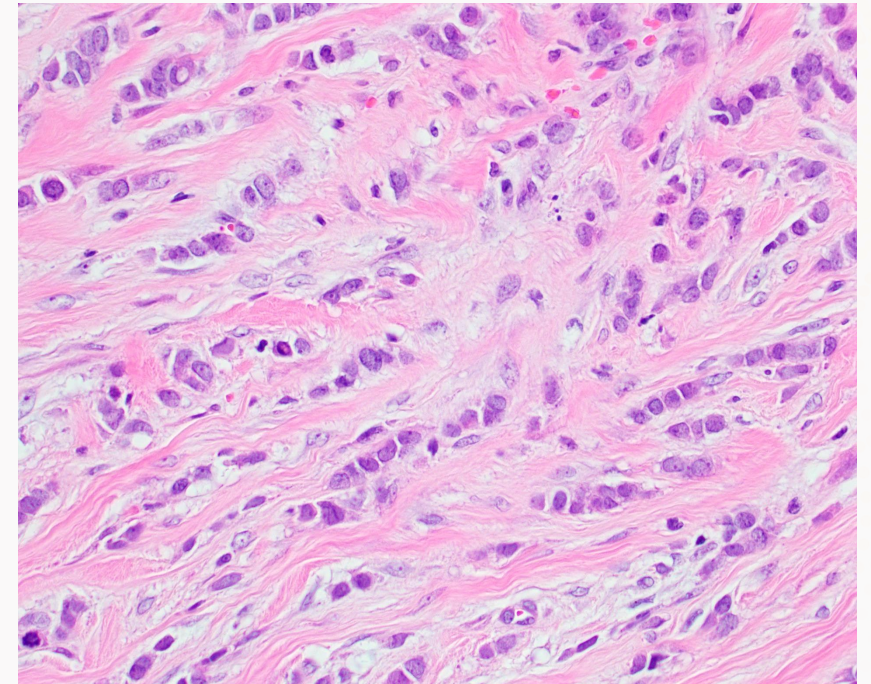
- MPhil at UQ
  - Prof Sunil Lakhani and A/Prof Amy McCart Reed
  - ROS1 as a potential predictive biomarker in ILC
- Clinical fellow with Breast Cancer Trials (BCT)
  - Largest independent oncology clinical trials group in Australia and New Zealand
  - Opportunity for early career researchers from different subspecialty areas to undertake projects related to existing or potential future clinical trials with BCT
    - Funding for up to 2 years part-time



**DEVELOPING NEW  
TREATMENTS FOR  
INVASIVE LOBULAR  
CARCINOMA**

# Invasive lobular carcinoma

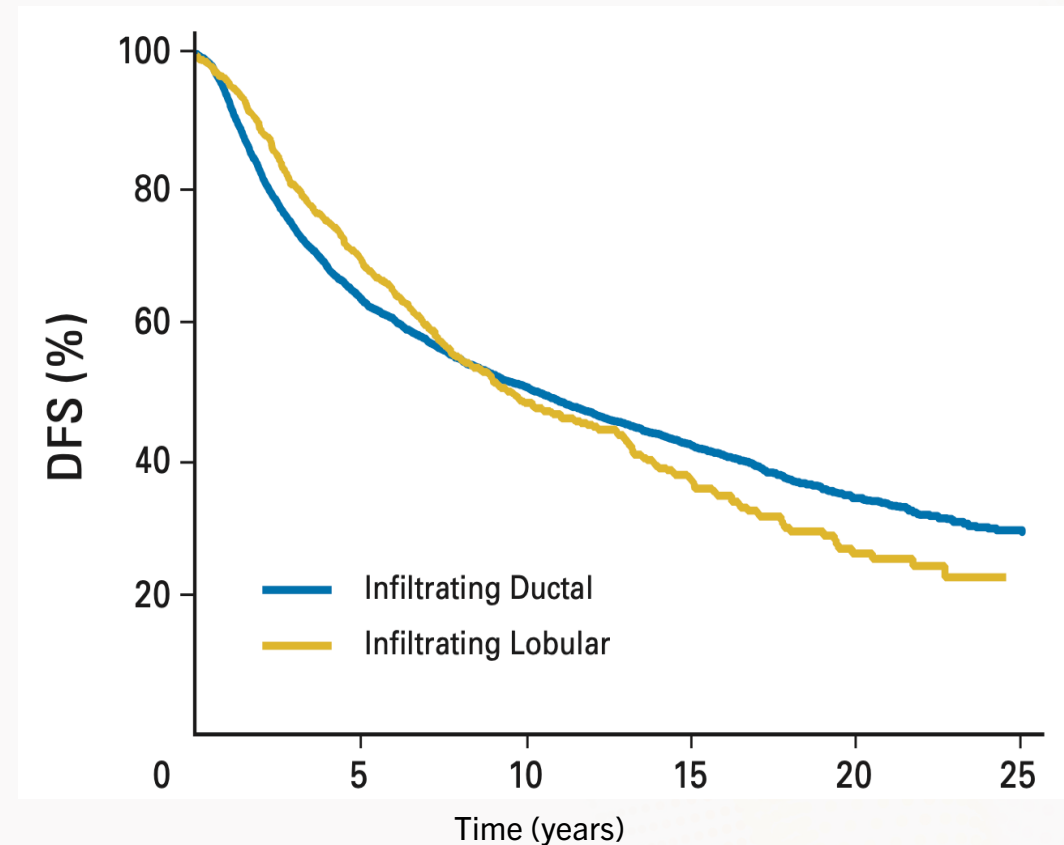
- Most common ‘special’ histologic subtype of breast cancer
- Characteristic tumour morphology
- *CDH1* alterations resulting in E-cadherin loss
- ER/PR positive, HER2 negative
- Luminal A



# ILC vs NST

- Clinical features
- Distinct molecular profile
- More resistant to chemotherapy
- More resistant to some types of endocrine therapy
- Unusual metastatic pattern
- Worse long-term prognosis

International breast cancer study group<sup>1</sup>

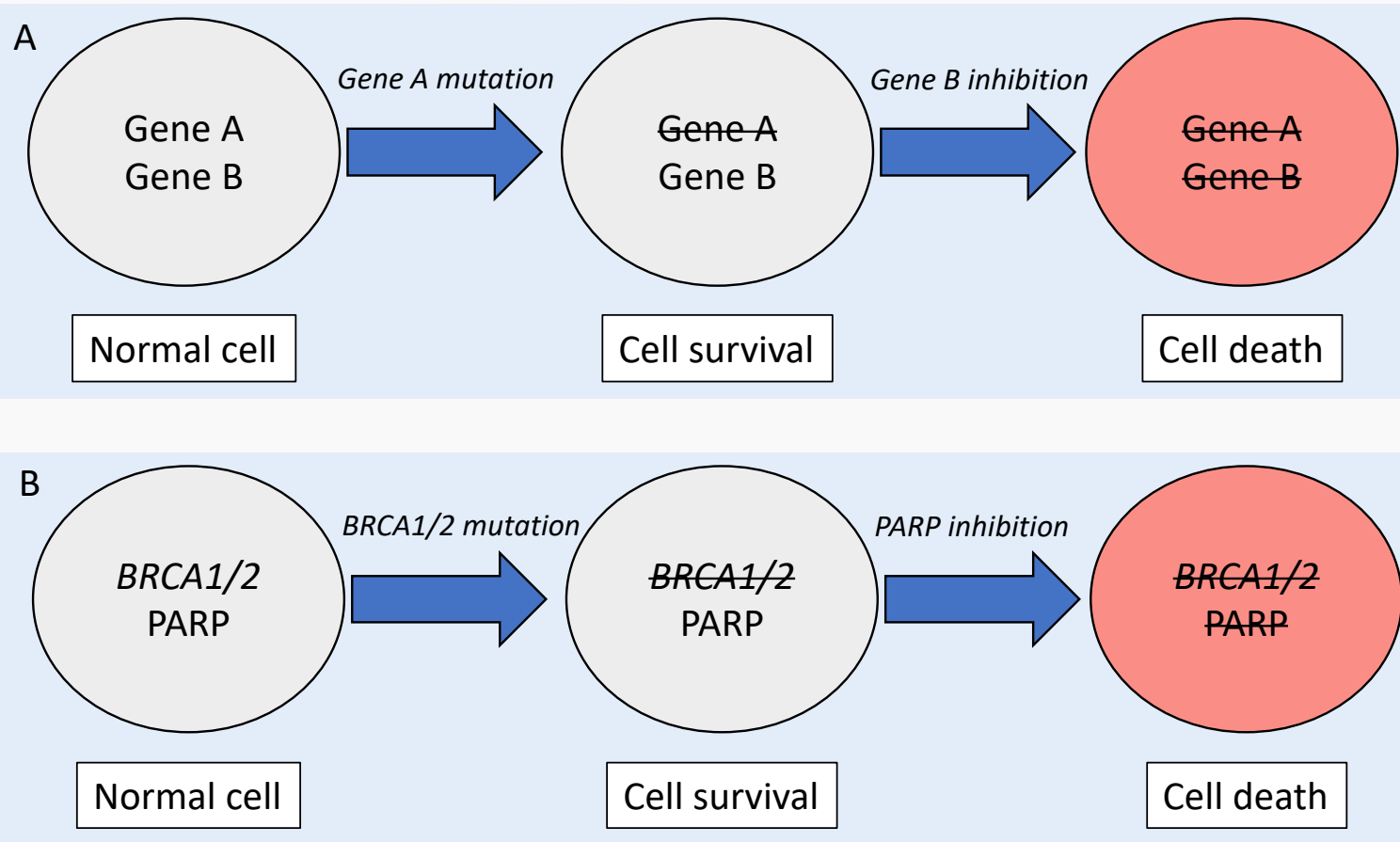


# Improving outcomes for ILC patients

- Despite differences in tumour biology, clinical behaviour and treatment response - ILC and NST are treated as the same disease clinically
- No ILC-specific biomarkers guiding treatment decisions
- ILC patients not well represented in clinic trials

*Need for translational research aimed at improving ILC outcomes*

# Synthetic lethality



- A. Therapeutic strategy targeting two interdependent genes
- B. *Synthetic lethality in clinical practice:* inhibition of PARP enzymes in hereditary breast cancers with germline *BRCA1/2* mutations results in targeted tumour cell death due to concurrent dysfunction of both DNA repair pathways

# E-cadherin/ROS1 synthetic lethality

*Cancer Discov.* 2018 April ; 8(4): 498–515. doi:10.1158/2159-8290.CD-17-0603.



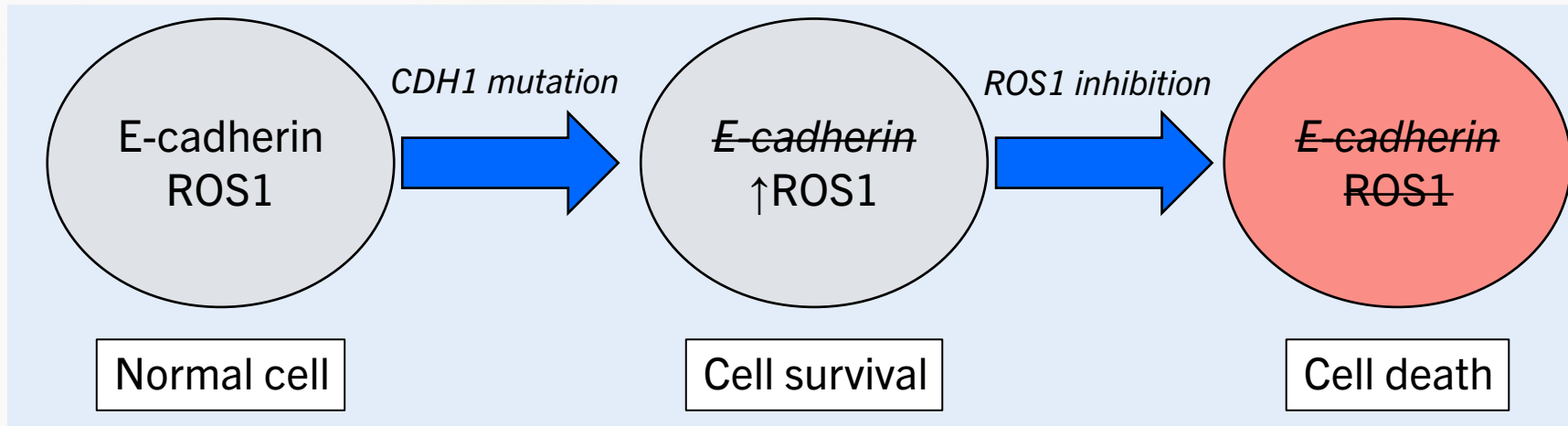
Europe PMC Funders Author M

## E-cadherin/ROS1 inhibitor synthetic lethality in breast cancer

Ilirjana Bajrami<sup>1,2</sup>, Rebecca Marlow<sup>3</sup>, Marieke van de Ven<sup>4</sup>, Rachel Brough<sup>1,2</sup>, Helen N. Pemberton<sup>1,2</sup>, Jessica Frankum<sup>1,2</sup>, Fei Fei Song<sup>1,2</sup>, Rumana Rafiq<sup>1,2</sup>, Asha Konde<sup>1,2</sup>, Dragomir B. Krastev<sup>1,2</sup>, Malini Menon<sup>1,2</sup>, James Campbell<sup>1,2</sup>, Aditi Gulati<sup>1,2</sup>, Rahul Kumar<sup>1,2</sup>, Stephen J. Pettitt<sup>1,2</sup>, Mark D. Gurden<sup>1</sup>, Marta Llorca Cardenosa<sup>1,5</sup>, Irene Chong<sup>1</sup>, Patrycja Gazinska<sup>3</sup>, Fredrik Wallberg<sup>6</sup>, Elinor J. Sawyer<sup>7</sup>, Lesley-Ann Martin<sup>1</sup>, Mitch Dowsett<sup>1</sup>, Spiros Linardopoulos<sup>1,8</sup>, Rachael Natrajan<sup>1</sup>, Colm J. Ryan<sup>9</sup>, Patrick W.B. Derksen<sup>10</sup>, Jos Jonkers<sup>11</sup>, Andrew N.J. Tutt<sup>1,3</sup>, Alan Ashworth<sup>12,\*</sup>, and Christopher J. Lord<sup>1,2,\*</sup>

ROS1 inhibitors (e.g. crizotinib) effective in multiple cancer models with defective E-cadherin (e.g. ILC, diffuse gastric cancer) through synthetic lethality

# E-cadherin/ROS1 synthetic lethality



*ROS1 inhibition in E-cadherin negative tumour cells caused mitotic failure and cell death*

- ROS1 is an established oncogenic driver in 1-2% NSCLC, rearrangements cause aberrant activation
- ROS1 may be a novel target in ILC and other tumours with defective E-cadherin
- Clinically actionable – ROS1 inhibitors already licensed in the lung cancer setting

# Current clinical trials (Phase II)

<b>ROLo</b>	<ul style="list-style-type: none"><li>• ROS1-targeted therapy (crizotinib) + endocrine rx</li><li>• Locally advanced or metastatic ILC and other <i>CDH1</i>-mutated tumours (diffuse gastric cancer)</li></ul>
<b>ROSALINE</b>	<ul style="list-style-type: none"><li>• Neoadjuvant ROS1-targeted therapy (entrectinib) + endocrine rx</li><li>• Locally advanced ILC</li></ul>

Primary outcome: Tumour response

Secondary outcomes: Adverse events, progression free and overall survival

Results expected mid-late 2024

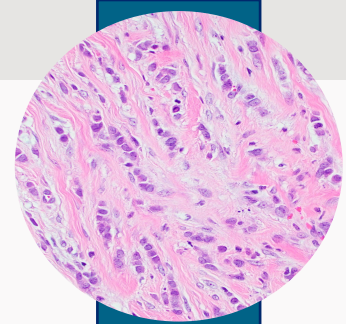
Third phase II clinical trial registered on [clinicaltrials.gov](https://clinicaltrials.gov) but not yet recruiting:

*Taletrectinib in Previously Treated Metastatic CDH1-mutated ILC*

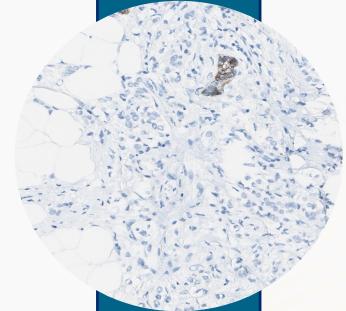
# ROS1 in breast cancer

- ROS1 expression has not been confirmed in ILC and ROS1 has not been validated as a biomarker in the breast cancer setting
- Implementing ROS1 as a predictive biomarker may facilitate treatment decisions
  - Identify which patients are likely to respond to ROS1 targeted therapy
  - Reduce prospective clinical trial failure by ensuring the trial population is enriched for patients who are likely to derive benefit from targeted treatment

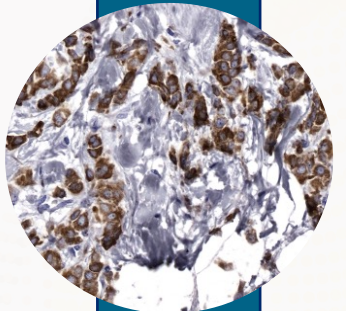
ILC



E-cad



ROS1



ROS1 inhibitors

# ROS1 expression in breast cancer

Authors	Year	N	BC subtype	Supplier / clone	Results
Eom et al	2013	203	NST	Santa Cruz biotechnology ?clone (discontinued)	54% ROS1 positive Correlated with ER positivity
Raut et al	2015	631	Unselected	Cell signaling technology D4D6 clone	All cases were ROS1 negative
Hameedi et al	2021	30	NST	BioSB EP282 clone	33% ROS1 positive Correlated with HER2 positivity

- Biological significance of ROS1 expression in breast cancer needs to be further clarified
- ROS1 expression has not been specifically characterised in ILC or other breast cancers with defective E-cadherin – relevant for identifying a patient cohort that is likely to respond to targeted treatment on the basis of synthetic lethality

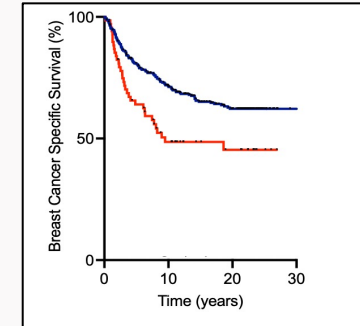
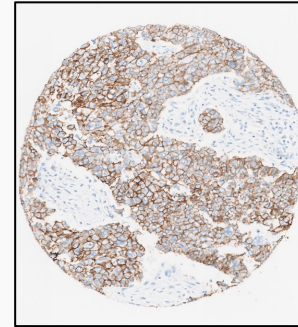
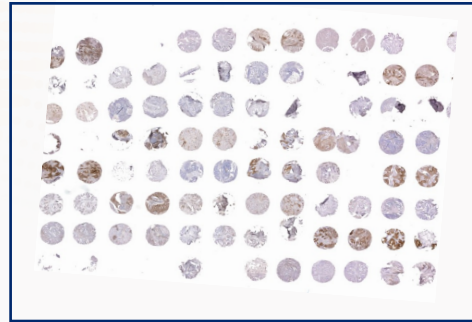
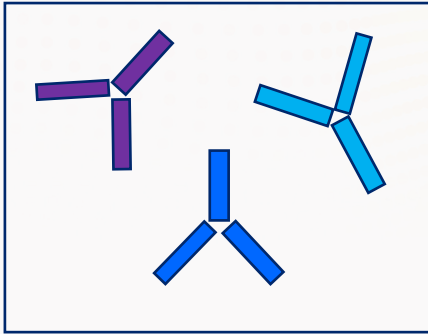
# Project aims

1. Examine ROS1 expression in a large well characterised cohort of breast cancer cases
2. Investigate the biology and clinical significance of ROS1 positivity in breast cancer
3. Determine whether ROS1 is a robust biomarker in breast cancer

# Materials and methods

- ✓ Clinical samples – TMAs constructed from FFPE tumour blocks
  - QFU cohort (668 unselected BC cases – 113 ILC, 30+ years follow up)
  - ILC focused cohort (216 ILC cases, 10+ years follow up)
- ✓ Antibodies for ROS1 IHC
  - Comparison of 4 different clones D4D6, SP384, EMPGHR2, EP282
- ✓ Control tissue
  - NSCLC with ROS1 rearrangement confirmed on FISH (positive control)
  - Commercial ROS1 control (positive and negative control)

# Project design



## IHC optimisation & validation

- Comparison of different ROS1 Ab clones

## Staining of TMAs

- 884 breast cancer cases across 12 TMAs

## IHC analysis

- Intensity (0, 1+, 2+, 3+), % of cells, pattern of staining, heterogeneity

## Statistical analyses

- Clinicopathological correlates
- Survival analyses

# Project outcomes

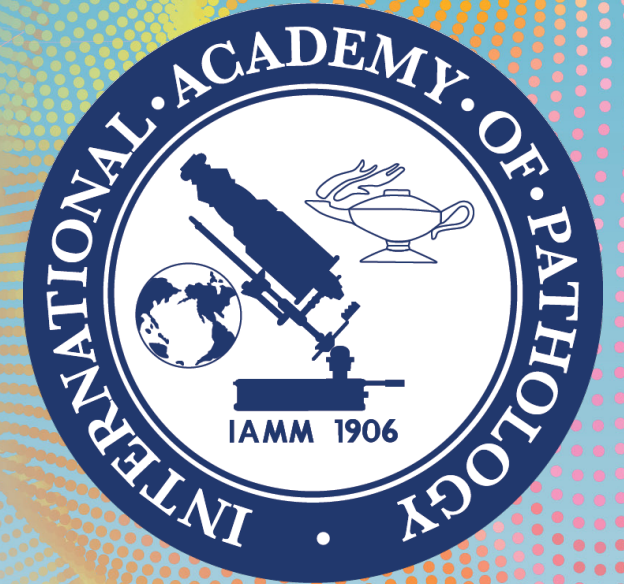
1. Inform the development of a clinically actionable biomarker for E-cadherin/ROS1 synthetic lethality in breast cancer
2. Determine how many breast cancer patients may benefit from ROS1-targeted therapy

# Thank you

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Prof Sunil Lakhani  
A/Prof Amy McCart Reed  
A/Prof Peter Simpson  
Dr Malcolm Lim  
Haarika Chittoory

Dr Jamie Kutasovic  
Dr Viabhavi Joshi  
Kaltin Ferguson  
Sullivan Nicolaides Pathology  
Breast Cancer Trials



## References

1. Pestalozzi BC, et al. Distinct clinical and prognostic features of ILC: combined results of 15 International Breast Cancer Study Group clinical trials. J Clin Oncol 2008; 26(18): 3006-14.