Recurrence Predictive Markers for DCIS

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- Introduction
- Controversies in DCIS
- Traditional predictors
- Molecular subtypes
- Biomarkers

Introduction

- Carcinoma in situ (CIS)
- Neoplastic proliferation of epithelial cells confined to ductal lobular units
- Low to high grade features
- Constitutes 15-30% of newly diagnosed breast cancer
- Infrequent prior to mammographic screening (76% detected by mammography)
- Inherent but not obligate tendency for progression to invasive disease
- Breast cancer specific mortality among women with DCIS:
  1.0-2.6% dying 8-10 years after initial diagnosis

SEER cancer statistics review 2012

Introduction

- DCIS are at risk for local recurrence (either as DCIS or invasive cancers)
  - Confers 8-10 times increased risk for further development to invasive cancer
- Present in up to 15% autopsy : women can die with asymptomatic DCIS without progression to invasive disease
- DCIS comprised of heterogeneous lesions that differ in their clinical presentation, pathological features, molecular markers and clinical course
- Some DCIS being over-diagnosed and over-treated?

Harris J et al Diseases of breast 2004
Page DL et al Cancer 1982

CHAPTER 5

Intraductal proliferative lesions

Introduction and overview

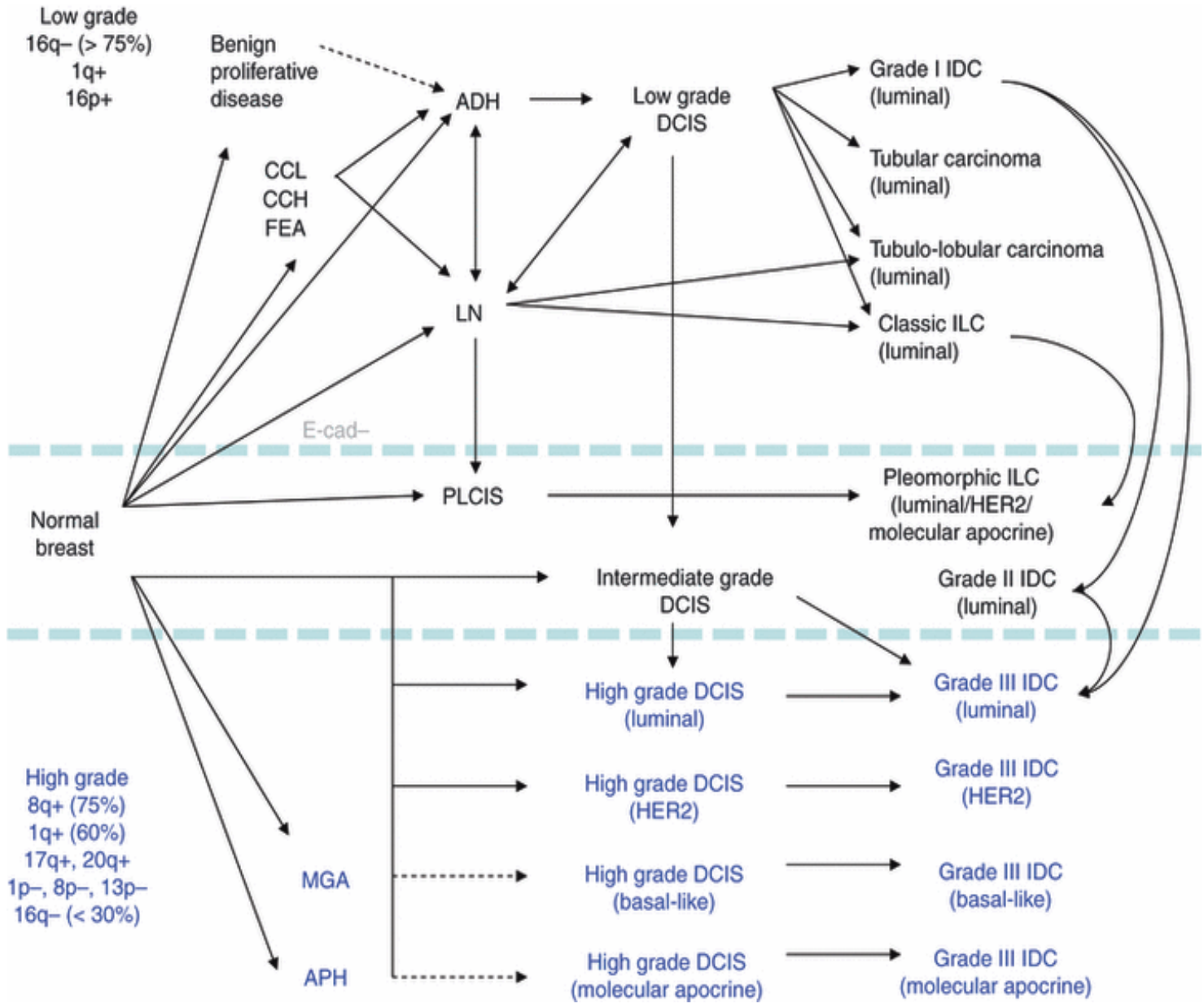
Usual ductal hyperplasia

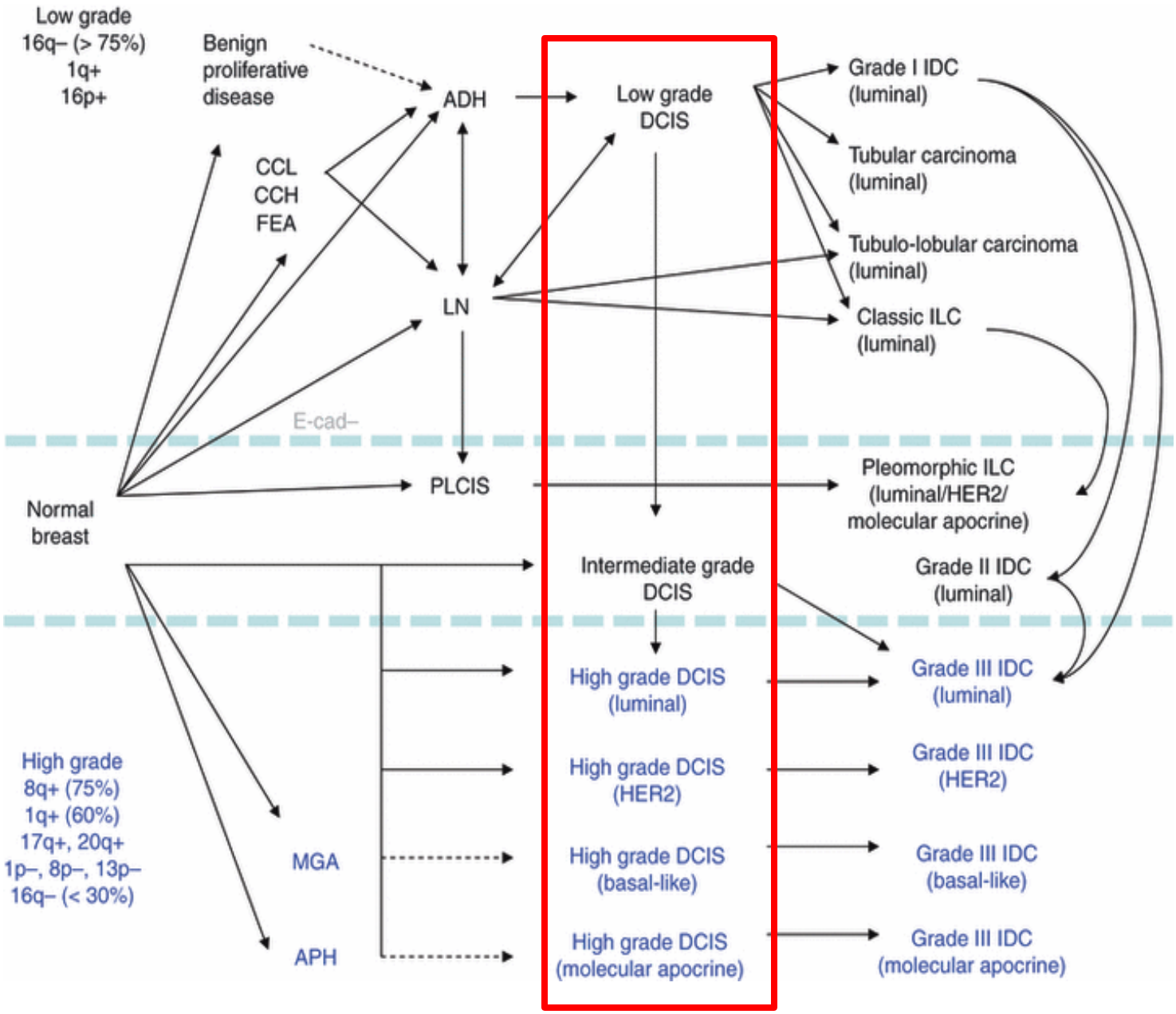
Columnar cell lesions

Atypical ductal hyperplasia

Ductal carcinoma in situ

WHO Tumour Classification, 2012


Low grade
16q– (> 75%)
1q+
16p+
Benign proliferative disease
ADH
Low grade DCIS
Grade I IDC (luminal)
Tubular carcinoma (luminal)
Tubulo-lobular carcinoma (luminal)
Classic ILC (luminal)
CCL
CCH
FEA
LN
E-cad–
PLCIS
Pleomorphic ILC (luminal/HER2/ molecular apocrine)
Normal breast
Intermediate grade DCIS
Grade II IDC (luminal)
High grade DCIS (luminal)
Grade III IDC (luminal)
High grade DCIS (HER2)
Grade III IDC (HER2)
High grade
8q+ (75%)
1q+ (60%)
17q+, 20q+
1p–, 8p–, 13p–
16q– (< 30%)
MGA
High grade DCIS (basal-like)
Grade III IDC (basal-like)
APH
High grade DCIS (molecular apocrine)
Grade III IDC (molecular apocrine)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Grade III IDC (molecular apocrine)

Intraductal proliferative lesions

Low grade arm

- FEA
- ADH
- DCIS low grade
- ALH/LCIS classical

High grade arm

- DCIS high grade
- Pleomorphic LCIS

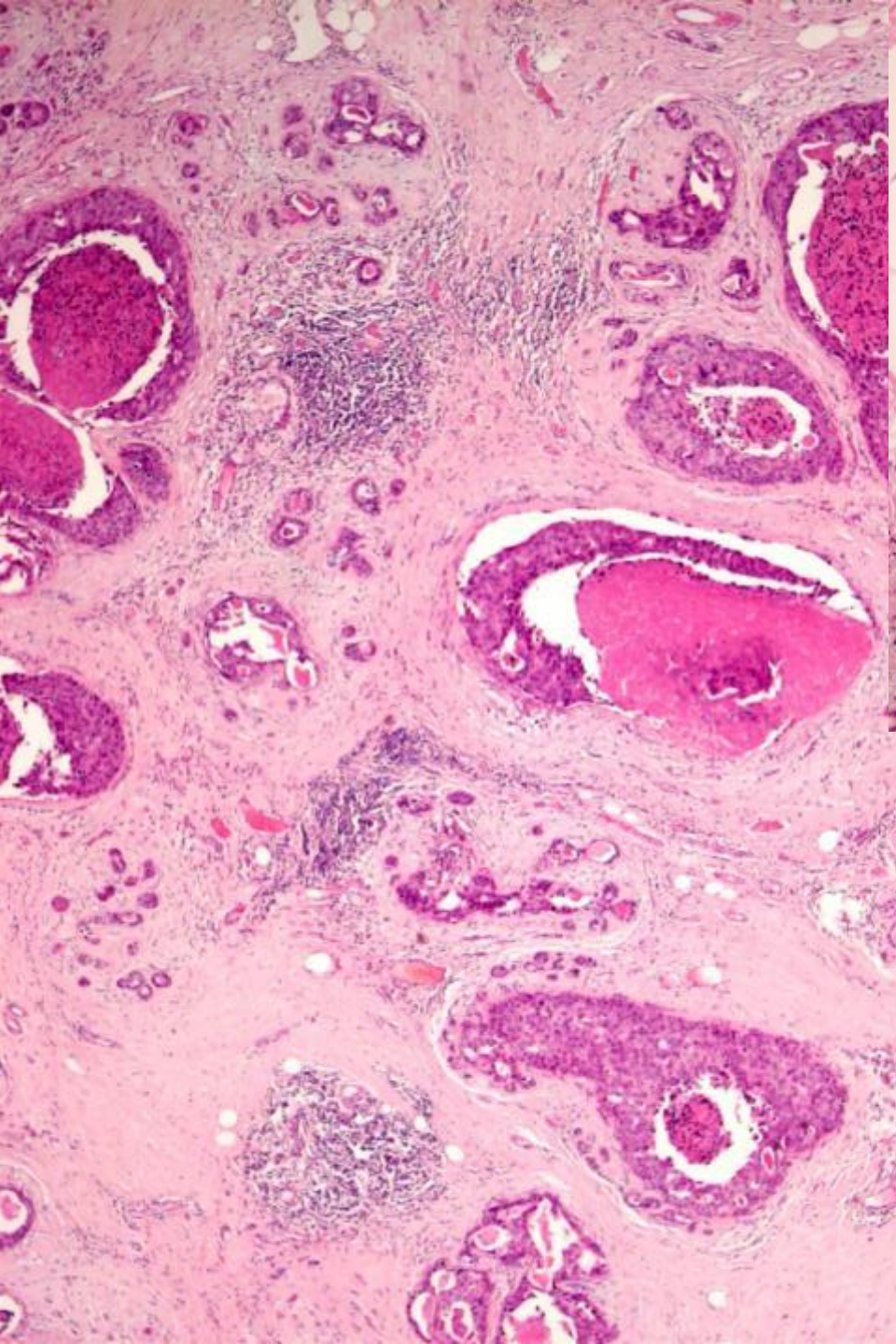
Intraductal proliferative lesions

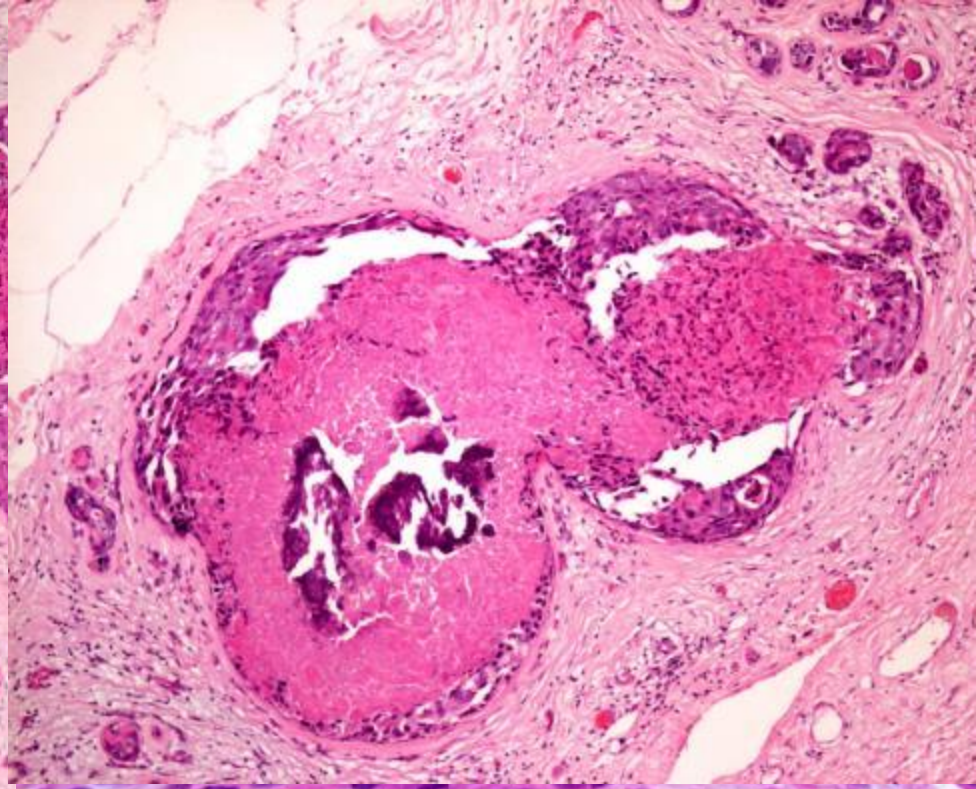
Low grade arm	High grade arm
• FEA	
• ADH	
• DCIS low grade	• DCIS high grade
• ALH/LCIS classical	• Pleomorphic LCIS

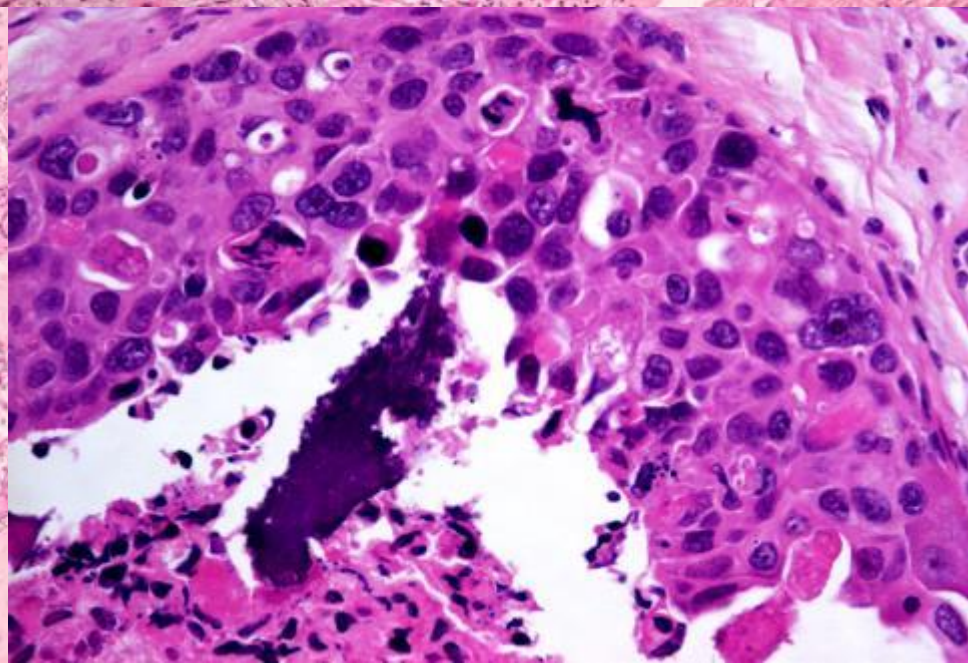
High grade DCIS

- HG DCIS
  - Amplification 17q12, 11q13
  - CGH showed similar changes in HG DCIS and invasive cancer : thus DCIS is an obligate precursor for invasive cancer
  - Gene profiling studies : similar groupings as to invasive cancers, HER2 and triple negative cases were mostly high grade

Ross DS et al. Adv Anat Pathol 2013,20:205-216



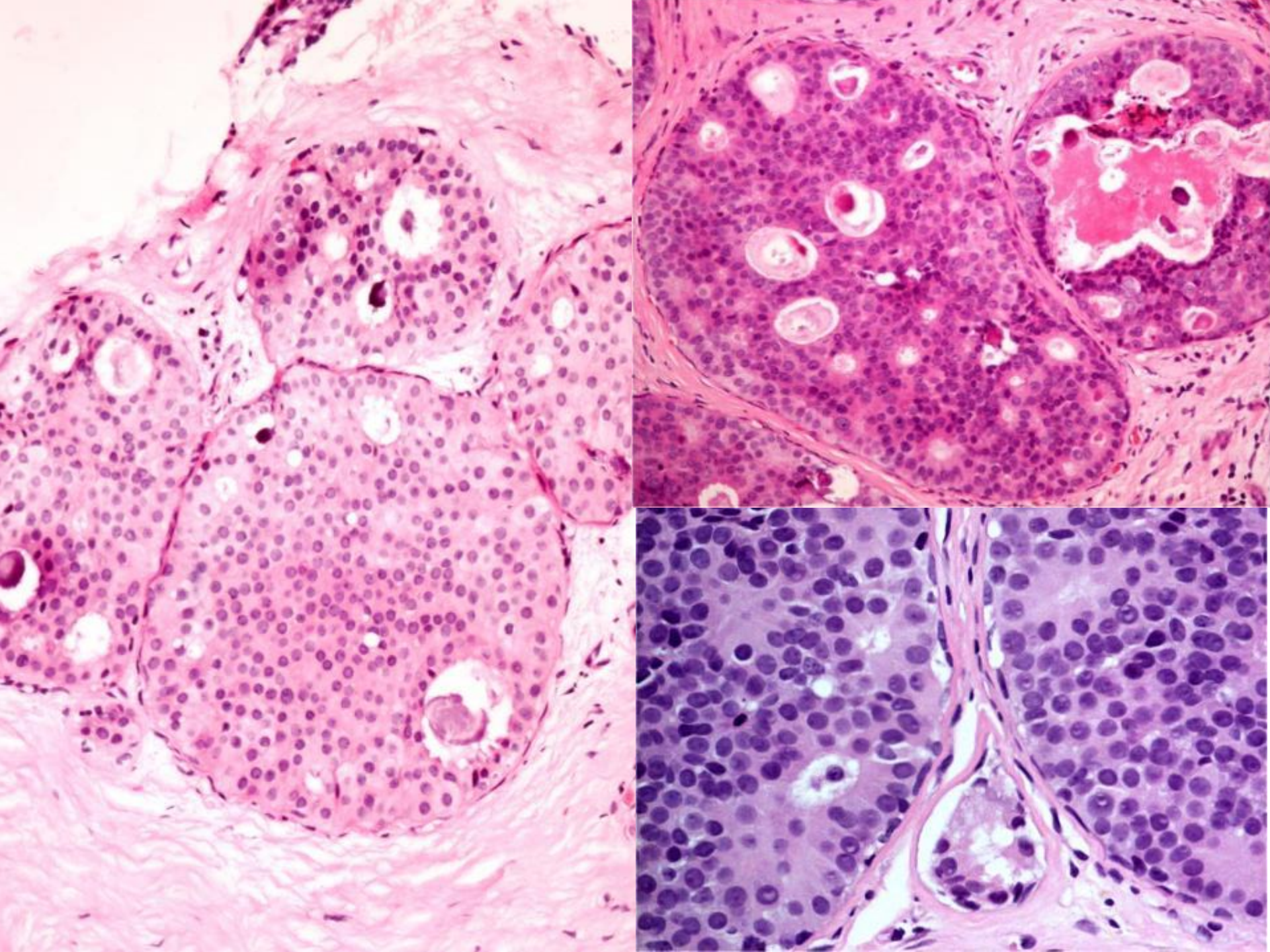




Low grade DCIS

- Monotonous cell population, rounded nuclei
- Geometric architecture
- Molecular changes
- More like to show 16q loss
- Low grade breast epithelial neoplasia
- Variable 1q gain
- Positive for ER, bcl2, cyclin D1, negative for HER2

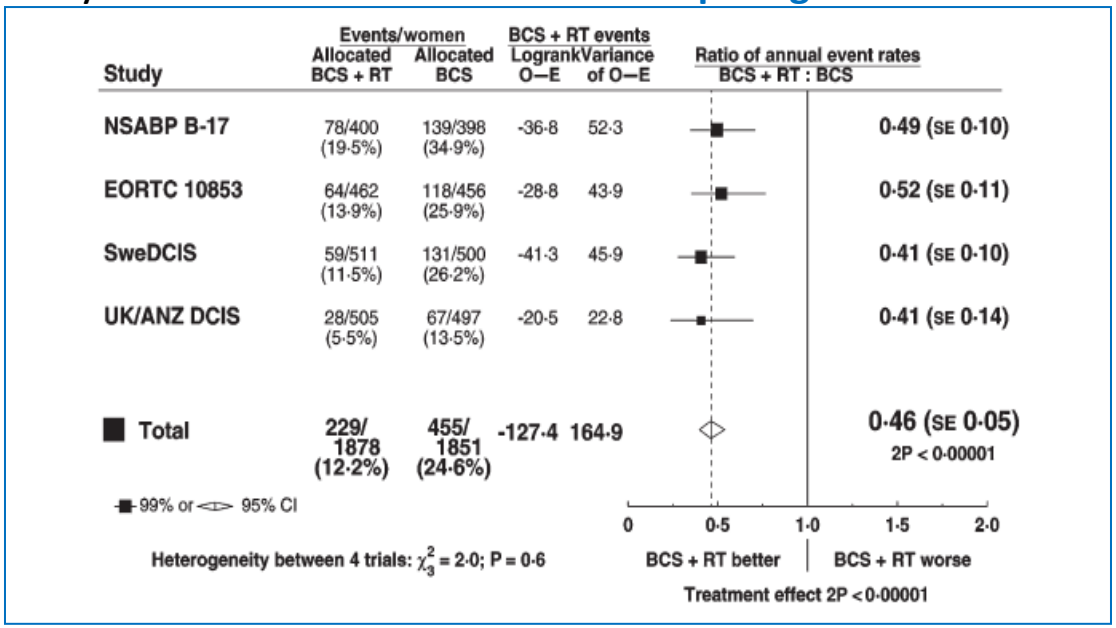
Abdel-Fatah TM et al. Am J Surg Pathol 2008;32:513
Ross DS et al. Adv Anat Pathol 2013,20:205-216



Controversy of DCIS treatment

- Treatment:
  - breast conserving surgery followed by radiotherapy
  - For ER+ DCIS, addition of tamoxifen to BCS and radiotherapy
- Addition of radiotherapy is associated with 50% reduction in rate of recurrence
  - 10 year absolute risk reduction: 15.2% irrespective of age, extent of BCS, detection method and histological factors
- Without radiotherapy, majority patients showed no recur
  - >70% in 10 years; 65% in 15 years

Randomized trials comparing BCS±RT



Early Breast Cancer Trialists' Collaborative Group (EBCTCG) 2010. *J Natl Cancer Inst Monogr.*

Controversy of DCIS treatment

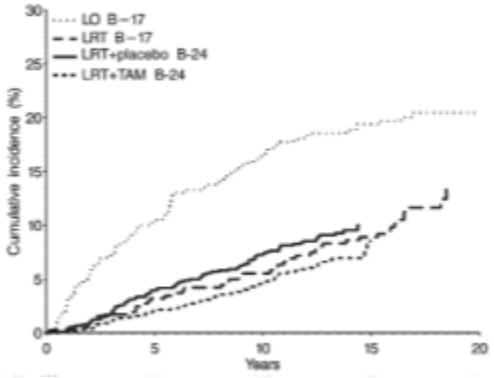
- Despite the reduced incidence of breast tumor recurrence, different treatments have little impact on patients' survival.

15 year results of NSABP B-17 trials (BCS±RT) and B-24 trials (BCS and RT±tamoxifen)

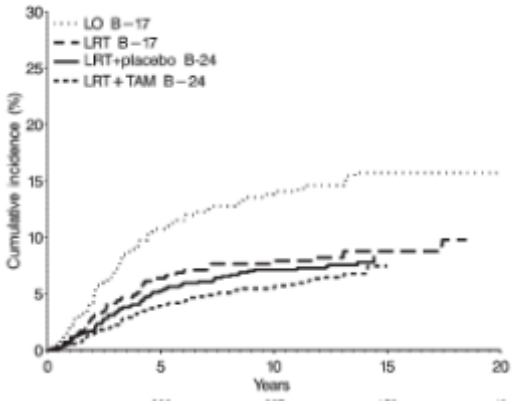
	B17 trials		B24 trial	
	BCS only (N=403)	BCS+RT (N=410)	BCS+RT (N=900)	BCS+RT+ Tamoxifen (N=899)
DCIS Recurrence	15.7%	8.8%	8.3%	7.5%
Invasive Recurrence	19.4%	8.9%	10.0%	8.5%
Breast cancer related death	3.1%	4.7%	2.7%	2.3%

Accumulative incidence of ipsilateral breast tumor recurrence

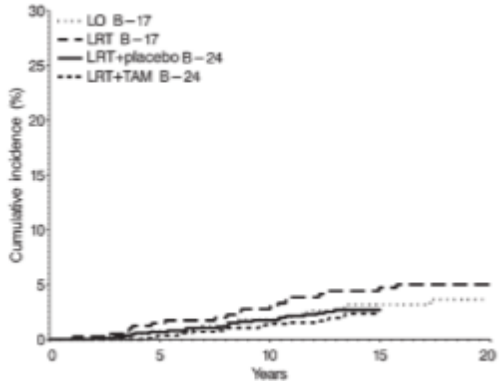
Invasive



DCIS



Accumulative incidence of breast cancer related death



Wapnir IL et al 2011 JNCI

Traditional predictors for recurrence risk of DCIS

- Based on patient and tumor characteristics

- Patient characteristics
  - Younger age (<40 years old)
  - Symptomatic DCIS

- Tumor characteristics (less standardized in term of definition)
  - Architectural subtypes
  - Nuclear grade
  - Presence/ absence of comedo necrosis
  - Size of lesions
  - Margin status

Architectural subtypes

Non-comedo (including cribriform, micropapillary, solid and papillary DCIS)

- Low grade cytology,
- ER+, no HER2 amp / p53 mutation

Comedo - more aggressive

- Mostly high grade, prominent central necrosis and calcification
- Frequently ER-, HER2 amp and p53 mutation
- High proliferation rate
- Angiogenesis
- Micro-invasion
- Higher rate of local recurrence

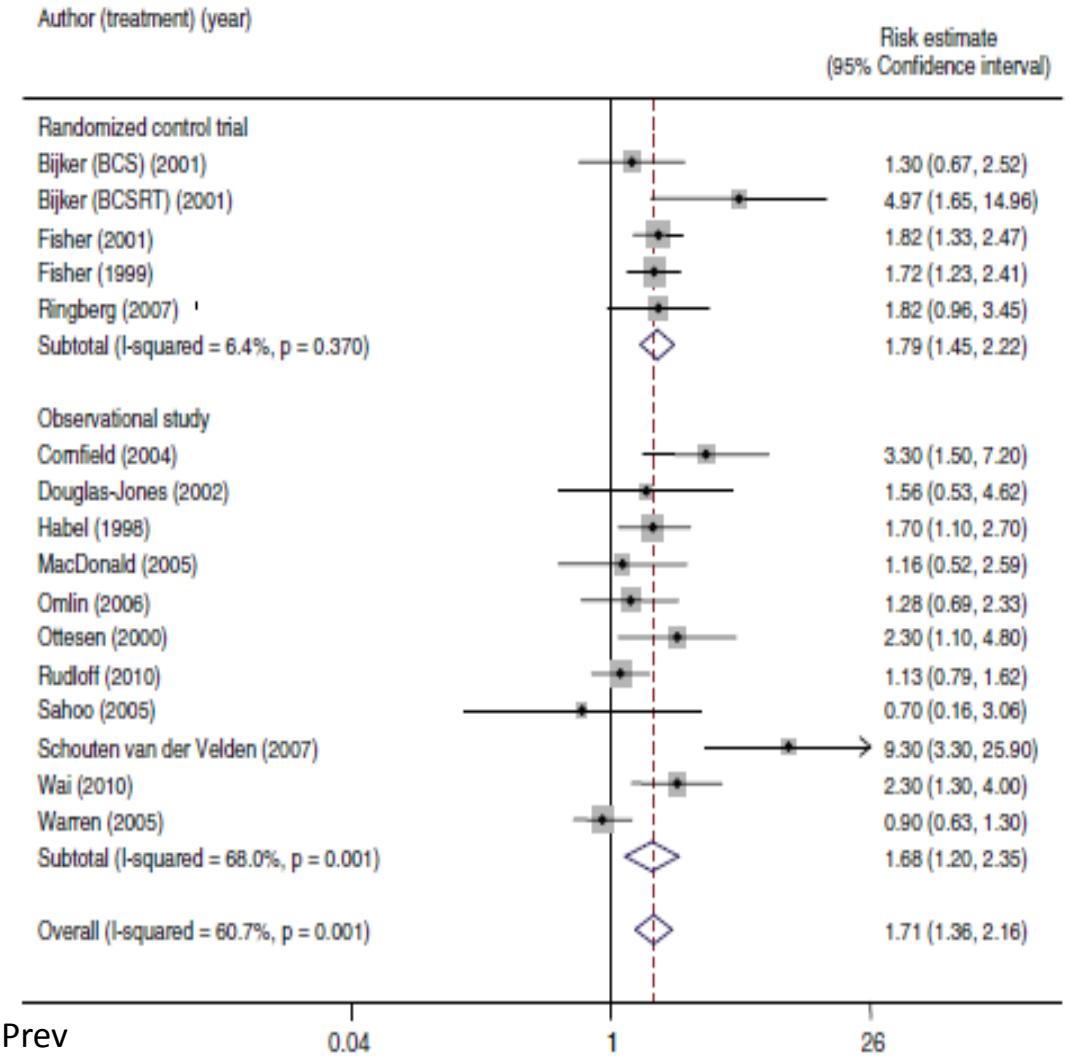
Problems

- Lesions most frequently show a mixture of architectures (62%)
- Individual duct space may show an architectural pattern that is difficult to categorize

Comedo Necrosis

- Rate of recurrence are generally higher for tumors with comedo necrosis than those without
- Weaker predictor of tumor recurrence than cellular architecture and nuclear grade
- High grade lesion with or without comedo necrosis showed similar biological behaviour

Meta-analysis on association between necrosis & ipsilateral cancer



Solin L et al 1993 Cancer
Wang S et al 2011 Breast Cancer Res Treat
Habel LA et al 1998 Cancer Epidemiol Biomarkers Prev

Nuclear grade

- Determined based on 6 features into grade I (low), II (intermediate) and III (high)

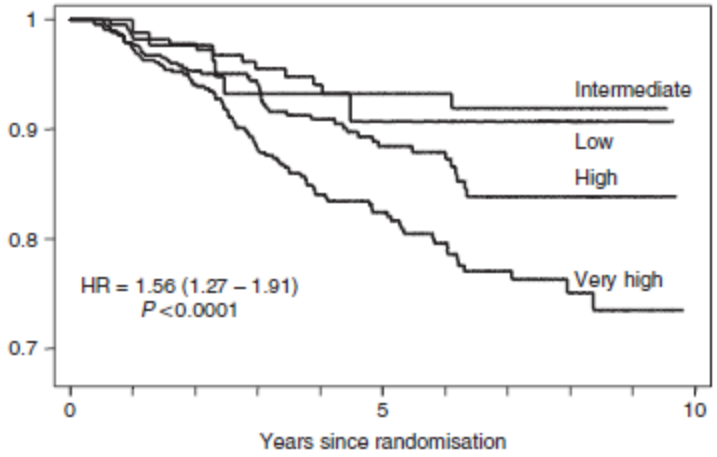
Features	Grade I	Grade II	Grade III
Pleomorphism	Monotonous	intermediate	Markedly pleomorphic
Size	1.5-2x the size of RBC or nucleus of normal ductal epithelial cells	intermediate	>2.5x the size of RBC or nucleus of normal ductal epithelial cells
Chromatin	Usually diffuse. Finely dispersed	intermediate	Usually vesicular and irregular chromatin distribution
Nucleoli	Only occasional	intermediate	Prominent, often multiple
Mitoses	Only occasional	intermediate	May be frequent
Orientation	Polarised toward luminal spaces	intermediate	Usually not polarised toward the luminal space

Schwartz GF et al Cancer 1997

- Less commonly shows a mixed pattern (15.7%) within individual lesion
- Genetic studies showed that low- and high- grade DCIS have different alterations suggesting that they are different groups of disease

Grading of DCIS with improved prediction of recurrence

- Results from UKCCCR/ANZ DCIS trials (involved 1694 cases) suggested that rates of recurrence did not differ between low and intermediate grade DCIS
- Reclassified as low/intermediate, high (<50% comedo) and very high (solid, >50% comedo)



Recurrence of ipsilateral DCIS/ invasive recurrence

Feature	Category	No. cases	No. events	HR (95% CI)	χ^2 for trend, P-value
Recurrence of ipsilateral DCIS or invasive					
Grading system, four tier	Low	86	6	0.42 (0.18–0.95)	22.11
	Intermediate	225	13	0.33 (0.19–0.60)	P=0.0001
	High	430	47	0.62 (0.43–0.88)	
	Very high	483	88	1.00[a]	
New grade, three tier	Low/intermediate	311	19	0.36 (0.22–0.58)	21.91
	High	430	47	0.62 (0.43–0.88)	P=0.0001
	Very high	483	88	1.00[a]	

Pinder SE et al 2010 BJC

Lesion size

- Extent of lesion can be ranged from 0.1 cm to involvement of all four quadrants
- No standardized definition for measuring the size of DCIS in published studies
- CAP guideline (2009)
  - Assessing size from one slide only if DCIS in one block; otherwise serial sequential sampling method
- Generally, <20mm as small tumor
- Meta-analysis including 7097 women with DCIS showed a62-68% increase in risk for patients with larger tumor

Lester S et al 2009 Arch Pathol Lab Med
Wang S et al 2011 Breast Cancer Res Treat

Margin status

- Determined by direct measurement between the smallest distance between the edge of the tumor and an inked line delineating the margin of normal tissue
- CAP guideline (2009) categorized as
  - Free: >0.2 cm from DCIS lesions
  - Close: 0.1-0.2 cm
  - Involved:<0.1 cm or DCIS is cross-sectioned
    - Focal: DCIS is present at a margin in <0.1cm area in one block
    - Extensive: DCIS is present at an area >1.5cm or ≥ 5 LPF and/or ≥ 8 blocks
    - Minimal/ moderate: between focal and extensive
- However, considerable variation across studies in terms of how margins were defined

Lester et al 2009 Arch Pathol Lab Med

Margin status

- Wider negative margin associated with reduced risk of recurrence regardless of RT
- Compared to negative margin >2mm, negative margin of 10mm were associated with a lower risk of recurrence

Table 3. Predicted probabilities of IBTR stratified by margin threshold and treatment*

		Margin threshold			
Treatment	Positive margin, mean (95% CI)	0 mm, mean (95% CI)	2 mm, mean (95% CI)	5 mm, mean (95% CI)	10 mm, mean (95% CI)
BCS plus RT	20% (16 to 24), N = 698	10% (8 to 13), N = 2057	9% (6 to 11), N = 742	11% (1 to 20), N = 23	4% (3 to 6), N = 86
BCS alone	35% (29 to 41), N = 423	20% (16 to 23), N = 1262	17% (12 to 22), N = 163	20% (3 to 36), N = 10	9% (5 to 12), N = 421

* BCS = breast-conserving surgery; CI = confidence interval; IBTR = ipsilateral breast tumor recurrence; RT = radiotherapy. The predicted probabilities of IBTR were estimated by the frequentist nonlinear mixed-effects model controlling for threshold and treatment status. All statistical tests were two-sided.

- Meta-analysis of 21 studies with total 1066 recurrence occurred in 7564 patients
- (565 IBTR in 3098 patients treated with BCS alone and 501 IBRT in 4466 patients with BCS +RT)

Wang S et al 2012 JNCI

Summary of tumor recurrence risk according to tumor characteristic and study design from meta-analysis

Tumor features	RCT/ no of studies	Observational studies/ no of studies	Overall	Level of confidence
Comedonecrosis (yes vs. no)	**1.79 (1.45, 2.22)/5***	**1.68 (1.20, 2.35)/11**	**1.71 (1.36, 2.16)**	High
Focality (yes vs. no)	**1.96 (1.55, 2.48)/5***	2.46 (0.87, 6.9)/2*	**1.95 (1.59, 2.40)***	Moderate
Margin (positive vs. negative)	**1.47 (1.12, 1.94)/5**	**2.84 (2.07, 3.89)/15**	**2.25 (1.77, 2.85)**	High
Method of detection (symptomatic vs. no)	**1.68 (1.38, 2.04)/4***	1.16 (0.91, 1.48)/7*	**1.35 (1.12, 1.62)***	Moderate
Grade (high vs. low)	**1.63 (1.30, 2.05)/6***	**1.99 (1.56, 2.52)/12***	**1.81 (1.53, 2.13)***	High
(intermediate vs. low)	**1.78 (1.26, 2.51)/2***	**1.79 (1.27, 2.53)/6***	**1.79 (1.40, 2.28)***	
Tumor size (large vs. small)	**1.62 (1.27, 2.06)/4***	**1.68 (1.12, 2.51)/9**	**1.63 (1.30, 2.06)**	High

Bold: statistical significance
*non-significant heterogeneity
RCT: randomized control trial

Wang S et al 2011 Breast Cancer Res Treat

Van Nuys Prognostic index

- VNPI - incorporate independent predictors for recurrence
- Score (size, margin, necrosis, nuclear grade and age)
- To achieve a local recurrence rate of <20% at 12 years
  - Score 4-6 : excision alone
  - Score 7-9 : excision plus radiotherapy
  - Score 10-12: Mastectomy
  - Fine tuning of treatment suggestions in 2010 :
    - Score 7 with ≥ 3mm margin: excision alone
    - Score 8 and 9 with <3 and 5 mm margin respectively: Mastectomy

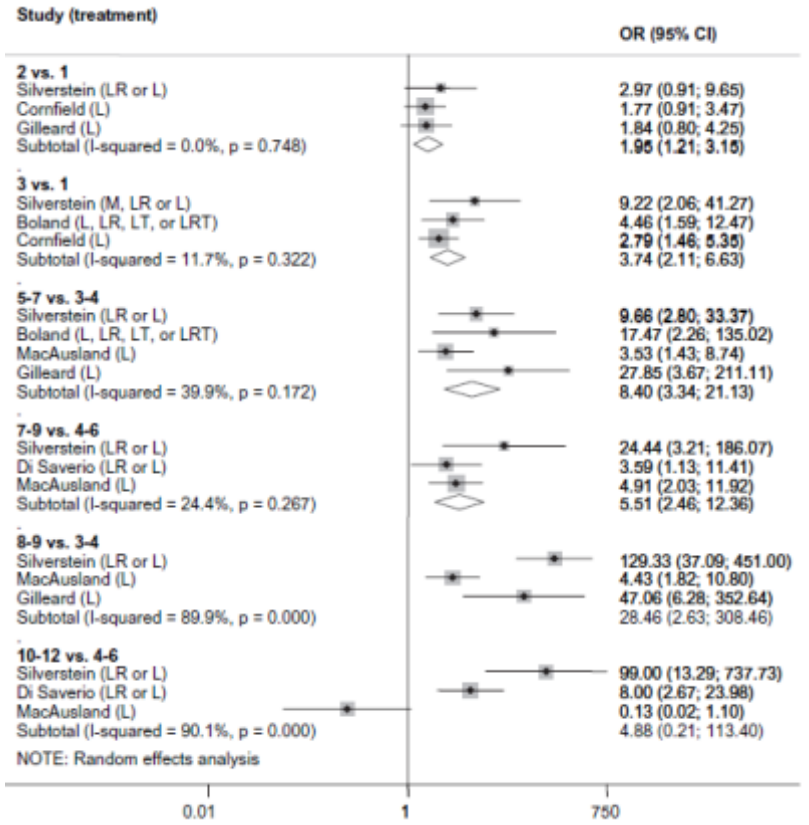
Score	1	2	3
Size	≤15mm	16-40	>40
Margin	≥10mm	1-9	<1
Pathological Classification	Grade 1 or 2 without necrosis	Grade 1 or 2 with necrosis	Grade 3
Age	>60	40-60	>40

Silverstein MJ and Lagios MD 2010 JNCI monogr

Van Nuys Prognostic index

- Meta-analysis showed a trend of association with higher risk score with higher rate of recurrence
- Comparing between different risk scores, some inconsistence between different studies.
- Further validation is required with large independent studies

Meta-analysis on association between VNPI with ipsilateral cancer



Shamliyan T et al 2010 JNCI monogr

DCIS and invasive recurrence

- Only invasive recurrence pose a serious threat to patient life
- Clinico-pathological risk factors differed for DCIS and invasive recurrence
- Clinico-pathological factors only associated with moderate increase of recurrence risk, particularly invasive recurrence.
  - Need to identify stronger risk factors to predict recurrence

Different clinico-pathological factors associated with increased risk of invasive and DCIS recurrence

	Recurrence	Age	Detection	Size	Margin	Grade	Comedo necrosis	Ref
• N=2995 • Median FU=4.8 yrs • Treatment= Breast conserving therapy • Multivariate analysis		Ref :65+	Ref: Mammography	*Ref: 1 LPF with DCIS	*Ref: ≥3mm			Collins et al 2013 Breast Cancer Res treat
	DCIS (N=172)	<45 (HR=2.0);	Symptoms (HR=1.6)	10-14 (5.1); 15-19 (6.5); 20+ (4.1)	<1 (HR=3.0); pos(HR=4.7); uncertain (HR=3.1)			
	Invasive (N=153)	<45 (HR=2.1)	Symptoms (HR=2.0)		Uncertain (HR=3.4)			
• N= 813 ; 1799 • Median FU= 17.25;13.6 yrs • Treatment= lumpectomy ±radiotherapy (LRT); LRT±tamoxifen • NASBP B17 and B24 trials • Univariate analysis		Ref:65+	Ref: mammography		Ref:LRT free	ND	Ref: absence	Wapnir et al 2011 JNCI
	DCIS (N=99; 128)	<45 (HR=2.9); 45-54 (HR=1.8); 55-64 (HR=1.7)	Clinically detected (HR=1.5)	ns	LRT pos/uncertain (HR=1.7)		Presence (HR=2.21)	
	Invasive (N=123; 137)	<45 (HR=2.1); 45-54 (HR=1.8); 55-64 (HR=1.5)	Clinically detected (HR=1.4)	ns	LRT pos/uncertain (HR=2.6)			
• N=1162 • Median FU= 8.2 yrs • Treatment= lumpectomy alone • Univariate analysis		ND	ND	Ref: ≤10mm	*Ref: ≥10mm	*Ref: low	Ref: moderate/scan t	Kerlikowske K et al 2010 JNCI
	DCIS (N=109)			>10 (HR=1.4)	2-10 (HR=2.3); 1-1.9 (HR=2.5); pos (HR=2.7); uncertain (HR=2.9)	High (HR=2.7); intermediate (HR=1.4)	Extensive (HR=1.5)	
	Invasive (N=114)				Pos (HR=1.6)			

*Nested case control study; ND- not determined; ns- not significant

Molecular subtypes in DCIS

- Risk of recurrence for invasive cancers can be predicted from molecular subtypes
- DCIS as non-obligate precursor to invasive cancers
- Similar molecular subtypes were observed in DCIS as invasive cancers by immunohistochemistry

Overall prevalence of molecular subtypes in DCIS and invasive cancers

	Luminal A	Luminal B	HER2	TNBC
DCIS (%)	57-62	10-13	13-22	10-12
Invasive cancers (%)	58-75	11-16	3-6	11-20

Clark SE et al 2011 BJC; Kwan ML et al 2009 Breast Cancer Res;
Tamimi RM et al 2008 Breast Cancer Res; Carey LA et al 2006 JAMA

Comparison of molecular subtypes in DCIS and invasive tumors

	Luminal A	Luminal B	HER2	Basal	Unclassified
DCIS (%) N= 272	62.5	13.2	13.6	7.7	3.0
Low grade	92.8	3.6	0	3.6	0
Intermediate grade	79.0	10.9	4.3	4.3	1.5
High grade	33.0	18.9	29.3	13.2	5.6
Invasive cancers (%) N=2249	73.4	5.2	5.6	10.9	4.9
Well differentiated	95.8	1.4	0	1.4	1.4
Moderately differentiated	79.4	5.5	4.8	7.1	3.2
Poorly differentiated	56.8	4.5	9.6	22.3	6.8
P-value	0.002	<0.001	<0.001	0.15	0.15

- Molecular subtypes in DCIS showed similar association with tumor grade
- Compared to invasive cancers, more Luminal B and HER2 but less luminal A phenotypes in DCIS

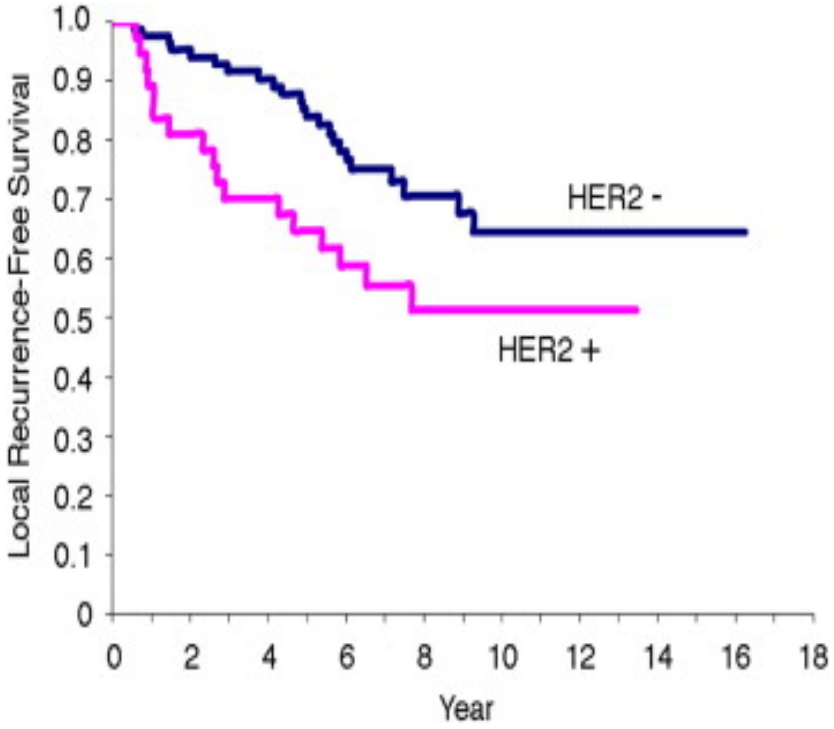
Tamimi RM et al. 2008 Breast Cancer Res

DCIS molecular subtypes and recurrence

- Luminal A DCIS has a lower risk to develop recurrence than luminal B
- HER2 positive DCIS had significant poorer recurrence free survival
- HER2 positivity was an independent predictor of increased recurrence risk

	Total	All recurrences (DCIS/ invasive)	Invasive recurrence
ER+ HER2-	106 (38.8%)	6 (5%)	2 (2%)
ER+ HER2+	85 (31%)	25 (29%)	6 (7%)
ER- HER2+	45 (16.5%)	15 (33%)	7 (16%)
ER- HER2-	37 (13.5%)	9 (14%)	5 (13.5%)
Total number	273	55	20
P-value		<0.01	<0.016

Williams KE et al 2012 San Antonio Breast Cancer Symposium



Han K et al 2012 Clin Oncol

Biomarkers and recurrence in DCIS

- Associated with increased risk of recurrence
  - p53, p21, Ki67, **HER2**, HER4, **defective RB pathway**
- Associated with decreased risk of recurrence
  - BCL-2, **ER** and PR
- Insignificant predictor
  - Cyclin D1, cathepin D, AR
- Many biomarkers did not show consistent risk association with recurrence or the evidence is based on single study

Barnes NL et al 2005 CCR, De Roos MA et al 2007 J Surg Res
Provenzano E et al 2003 EJC, Omlin A et al 2006 Lancet Oncol

HER2 predicts DCIS but not invasive recurrence

Table 4 Molecular predictors of non-invasive (DCIS) local recurrence

	N	No of DCIS LR	Hazard ratio (95% CI)	P-value
Univariable analysis				
HER2/neu+			2.72 (1.26, 5.88)	0.01
Psoriasin (⩾10%)			1.30 (0.52, 3.24)	0.57
Calgranulin (⩾10%)			1.47 (0.62, 3.49)	0.39
Ki67 (⩾10%)			1.05 (0.47, 2.35)	0.91
p53 (⩾10%)			0.89 (0.40, 1.99)	0.77
ER positive			1.14 (0.48, 2.71)	0.77
PR positive			0.71 (0.33, 1.53)	0.37
Cyclin D1 (⩾10%)			1.01 (0.99, 1.02)	0.52
p21 (⩾10%)			1.24 (0.57, 2.71)	0.58
Multivariable analysis (adjusted for age and XRT)				
Her2/neu+	58	13	2.67 (1.23, 5.79)	0.01
HER2/neu+/Ki67+ (*vs* other)	51	10	3.22 (1.47, 7.03)	0.003
HER2/neu+/Ki67− (*vs* other)	7	0	Not calculable	
HER2/neu+/p53+(*vs* other)	35	5	1.54 (0.61, 3.91)	0.36
Ki67+/p53+ (*vs* other)	63	6	1.09 (0.44, 2.67)	0.86
HER2/neu+/Ki67+/p53+ (*vs* other)	31	5	1.79 (0.70, 4.57)	0.22
ER−/HER2/neu+/Ki67+ (*vs* other)	31	6	1.65 (0.66, 4.15)	0.28

Table 5 Molecular predictors of invasive recurrence

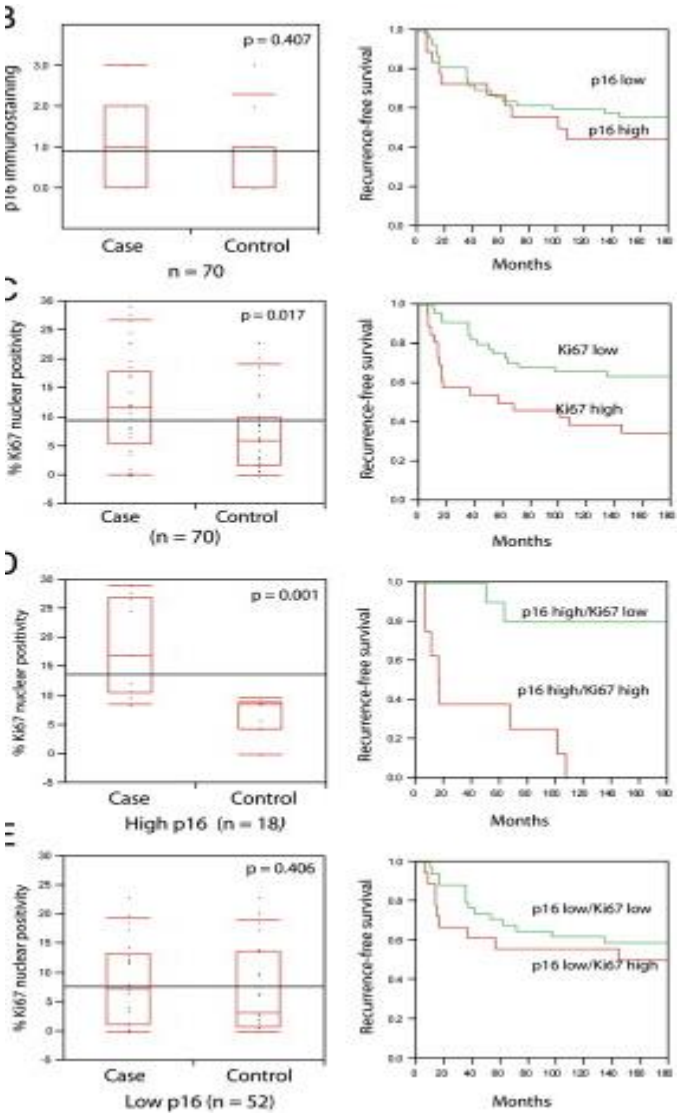
Univariable analysis	N	No of Inv LR	Hazard ratio (95% CI)	P-value
HER2/neu+			1.58 (0.69, 3.62)	0.28
Psoriasin (⩾10%)			0.38 (0.09, 1.60)	0.19
Calgranulin (⩾10%)			1.24 (0.49, 3.12)	0.65
Ki67 (⩾10%)			0.79 (0.35, 1.77)	0.56
p53 (⩾10%)			0.88 (0.38, 2.06)	0.77
ER positive			0.64 (0.29, 1.45)	0.29
PR positive			1.30 (0.55, 3.03)	0.55
Cyclin D1 (⩾10%)			0.99 (0.98, 1.01)	0.85
p21 (⩾10%)			0.85 (0.38, 1.90)	0.69
Multivariable analysis (adjusted for age and XRT)				
HER2/neu/neu+	58	9	1.61 (0.70, 3.73)	0.26
HER2/neu+/Ki67+ (*vs* other)	51	6	1.33 (0.54, 3.28)	0.54
HER2/neu+/Ki67− (*vs* other)	7	2	1.22 (0.29, 5.06)	0.79
HER2/neu+/p53+(*vs* other)	35	3	1.04 (0.35, 3.11)	0.94
Ki67+/p53+ (*vs* other)	63	6	1.41 (0.57, 3.52)	0.46
HER2/neu+/Ki67+/p53+ (*vs* other)	31	3	1.22 (0.40, 3.69)	0.73
ER−/HER2/neu+/Ki67+ (*vs* other)	31	5	1.39 (0.51, 3.78)	0.52

- 213 women treated with breast conserving therapy (72 with adjuvant radiotherapy)
- Rate of recurrence at 10 years was 36% for patients with surgery alone
- 18% for those with adjuvant radiotherapy

Rakovitch E et al 2012 BJC

p16+Ki67+ and recurrence

- Cellular senescence – a barrier for progression of DCIS to invasive cancer
- p16 overexpression can represent:
  - activation of response to cellular stress leading to senescence
  - loss of negative feedback due to abrogation of functional RB pathway
- Abrogation of functional RB leads to cell proliferation and bypass senescence, thus the cells will express high Ki67
- Loss of RB is an independent prognostic factor for recurrence free survival (Knudsen ES et al 2012 JNCI)
- P16 overexpression with high Ki67 can identify DCIS associated with recurrence (Gauthier ML et al 2007 Cancer cell; Witkiewicz AK et al 2011 AJPath)



Gauthier ML et al 2007 Cancer cell

Interaction of COX2 and p16 pathway

- DCIS with COX2+Ki67+ also showed shorter recurrence free survival as P16+Ki67+ DCIS
- High COX2 expression fall within the same gene cluster well established for basal like subtype as p16 expression
- Anti-tumorgenic activity of COX2 depends on p16/RB pathway
  - COX2 overexpression in cells with functional p16/RB signaling induced a p16-dependent growth arrest while cells with disrupted p16/RB signaling continue to proliferate in the presence of COX2 overexpression
- Silencing of Rb expression resulted in upregulation of COX2 expression

Combination of p16, Ki67 and COX2 for assessing risk of subsequent tumor

- 1162 DCIS patients - lumpectomy alone, FU period 9 yrs
- DCIS recurrence (154 patients); invasive recurrence (170 patients)
- individual markers ER, PR, p53, HER2 and COX2 were not statistically significantly associated with subsequent invasive tumor.

Factor†	No subsequent tumor event‡ (N = 186), % (No.)	Invasive event (N = 72), % (No.)	Risk of invasive event, HR§ (95% CI)	DCIS event (N = 71), % (No.)	Risk of DCIS event, HR§ (95% CI)
ER					
Negative	20 (35)	20 (13)	0.8 (0.4 to 1.5)	31 (21)	1.7 (1.0 to 2.9)
Positive	80 (143)	80 (53)	1.0 (referent)	69 (47)	1.0 (referent)
PR					
Negative	21 (36)	31 (20)	1.3 (0.7 to 2.1)	33 (21)	1.5 (0.9 to 2.5)
Positive	79 (138)	69 (45)	1.0 (referent)	67 (42)	1.0 (referent)
p53					
Positive	10 (17)	10 (6)	0.8 (0.4 to 1.9)	17 (10)	1.8 (0.9 to 3.5)
Negative	90 (153)	90 (57)	1.0 (referent)	83 (49)	1.0 (referent)
ERBB2 oncoprotein					
Positive	13 (25)	19 (14)	1.1 (0.6 to 1.9)	30 (21)	2.0 (1.2 to 3.2)
Negative	87 (161)	81 (58)	1.0 (referent)	70 (50)	1.0 (referent)
Ki67					
Positive‖	36 (62)	59 (38)	1.7 (1.0 to 2.7)	67 (40)	2.3 (1.3 to 4.1)
Negative	64 (109)	41 (26)	1.0 (referent)	33 (20)	1.0 (referent)
p16					
Positive	30 (43)	57 (37)	2.3 (1.4 to 3.8)	41 (26)	1.1 (0.7 to 1.8)
Negative	70 (98)	43 (28)	1.0 (referent)	59 (38)	1.0 (referent)
COX-2					
Positive	46 (68)	50 (34)	1.3 (0.8 to 2.0)	34 (22)	0.6 (0.4 to 1.1)
Negative	54 (79)	50 (34)	1.0 (referent)	66 (42)	1.0 (referent)

Kerlikowske K et al JNCI 2010

Multivariate analysis of biomarker expression and risk of tumor recurrence

Variable	HR (95% CI)	
	Recurrence as invasive cancer	Recurrence as DCIS
Age at diagnosis	1.0 (0.8-1.3)	0.9 (0.7-1.1)
Detection by palpation (vs mamography)	2.7 (1.4 to 5.5)	
Margins ordinal		1.3 (1.1 to 1.7)
Nuclear grade		
High vs low	1.0 (0.4 to 2.3)	1.7 (0.6 to 4.8)
Intermediate vs low	1.9 (0.8 to 4.3)	1.3 (0.4 to 4.1)
P16/COX-2/Ki67 (vs other groupings)		
+ve/+ve/+ve	2.2 (1.1 to 4.5)	
+ve/-ve/+ve		3.7 (1.7 to 7.9)
ER/ERBB2/Ki67 (vs other groupings)		
-ve/+ve/+ve		5.8 (2.4 to 14)

COX2 and p16 both fall into gene cluster of basal like tumor may share characteristic of Basal like invasive cancer

Biological role of COX2 in promoting tumor invasive potential;

COX2+ cancer tend to relate to invasive recurrence

Kerlikowske K et al JNCI 2010

DCIS score algorithm

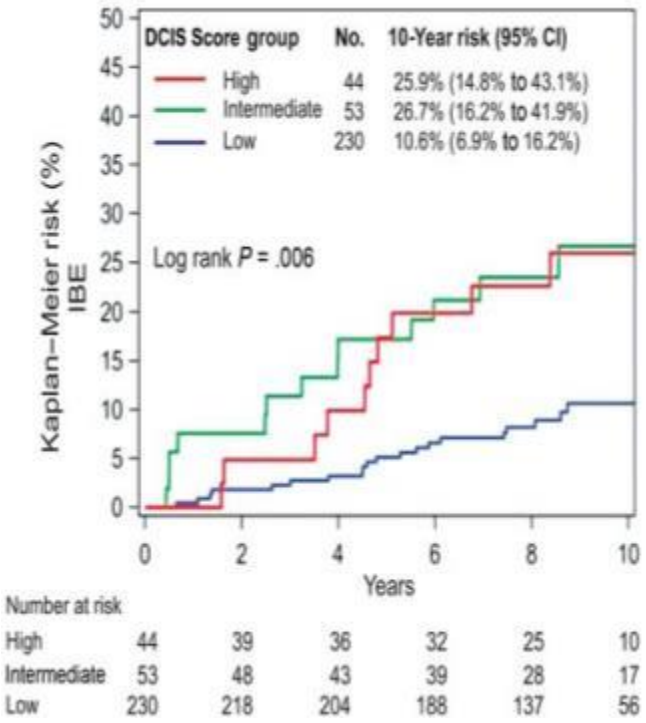
- Development based partly on quantitative expression of genes from the 21-gene Oncotype DX recurrence score for prediction of local recurrence
- Comparing adjacent DCIS and invasive components in same FFPE tumor blocks by 21-gene Oncotype DX from 30 cases(Baehner FL et al CTRC-AACR San Antonio Breast Cancer Symposium: 2008)
  - Not all DCIS had a low score and a strong correlation of recurrence scores between the pairs
  - High correlation (>0.73) of individual gene expression (except invasive genes and lower proliferation scores in DCIS) between the two components were observed.
  - ➢ More aggressive biology for IBC identified by recurrence score might also present in DCIS
- Similar results were found with comparing independent cohorts of 94 pure DCIS and 74 IBCs (Solin et al 2013 JNCI)
  - 90% DCIS showed low proliferation score <6.5 (threshold used in recurrence score for IBC)
  - ➢ Full range of proliferation group expression is used which provides important information for predicting local recurrence
- NSABP B-14 and Kaiser Permanente studies showed that only the proliferative gene group , PR and GSTM1 were prognostic for prediction of distant recurrence and breast cancer mortality for patients treated with tamoxifen as well as not treated with tamoxifen (Habel et al 2006 Breast Cancer Res)
  - Genes predictive for recurrence independent of Tamoxifen treatment are selected as tamoxifen use for DCIS is variable

DCIS score

- DCIS score algorithm includes
  - Proliferation: Ki67, STK15 (aurora kinase A), Survivin, Cyclin B1, MYBL2 (v-myb myeloblastosis viral oncogene homolog like 2)
  - Other cancer related genes: PR, GSTM1
  - Reference: β-actin, GAPDH, RPLPO, GUS, TFRC
- Similar to the 21 gene Oncotype DX score, the DCIS score is scaled as a continuous variable from 0-100
- Pre-specified DCIS score risk groups
  - Low: <39
  - Intermediate: 39-54
  - High: ≥55

10 year risk estimated by DCIS score

Any ipsilateral events

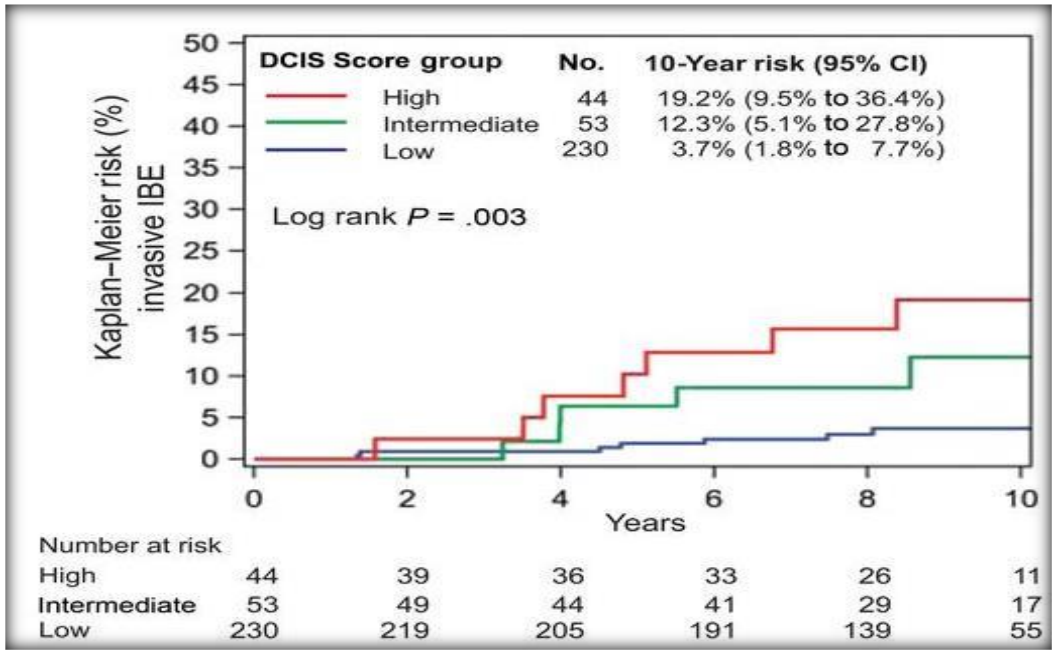


Pre-specified risk groups based on DCIS score

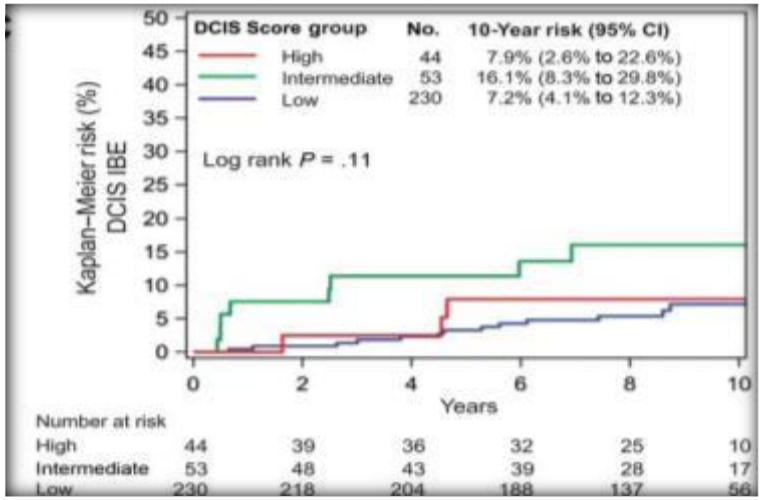
- High (≥55)
- Intermediate (39-54)
- Low (<39)

Solin LJ et al JNCI 2013

Recurrence as invasive cancer

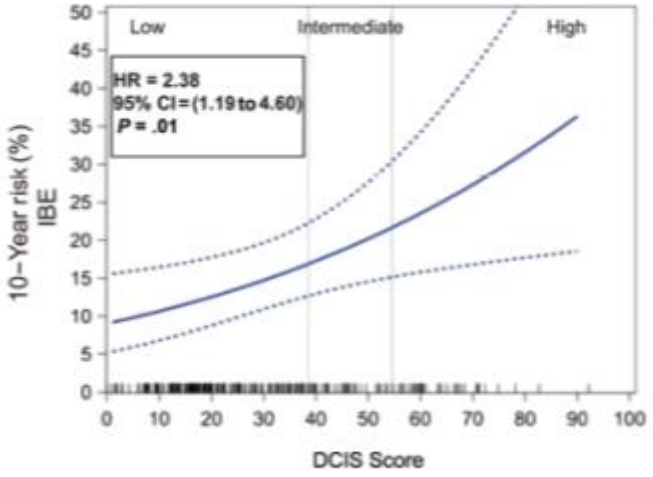


Recurrence as DCIS

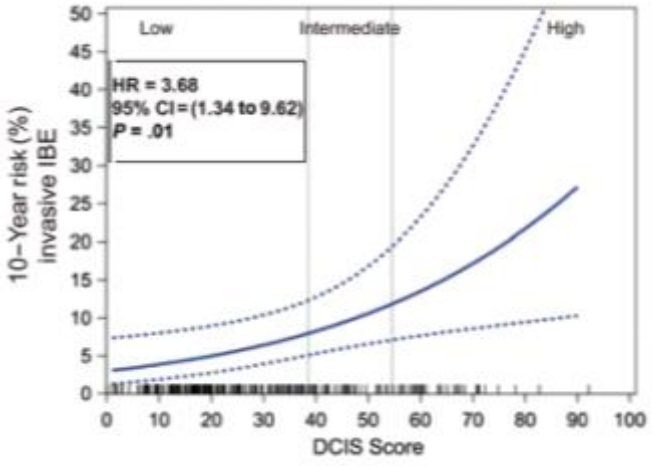


Estimated 10 year risk as a continuous function using the DCIS score

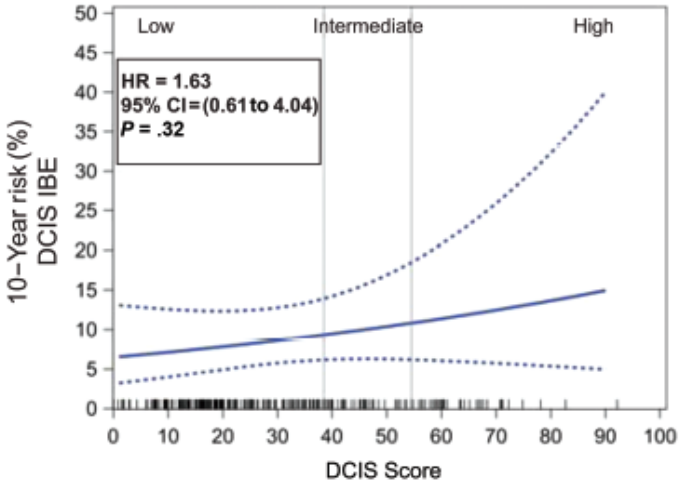
All ipsilateral recurrence



invasive recurrence



DCIS recurrence



Estimated risk of invasive recurrence from score 0 to 90

- All recurrence : 9% to 38%
- DCIS recurrence: 7% to 15%
- Invasive recurrence: 3% to 29%

Multivariate analysis for recurrence risk with clinico-pathological factors with/ without DCIS score

Variables	Hazard ratio (95% CI)	P-value
Excluding DCIS score		
Postmenopausal	0.49 (0.27-0.90)	0.02
Tumor size	1.54 (1.14-2.02)	0.006
Including DCIS score		
Postmenopausal	0.49 (0.27-0.90)	0.02
Tumor size	1.52 (1.11-2.01)	0.01
DCIS score	2.37 (1.14-4.76)	0.02

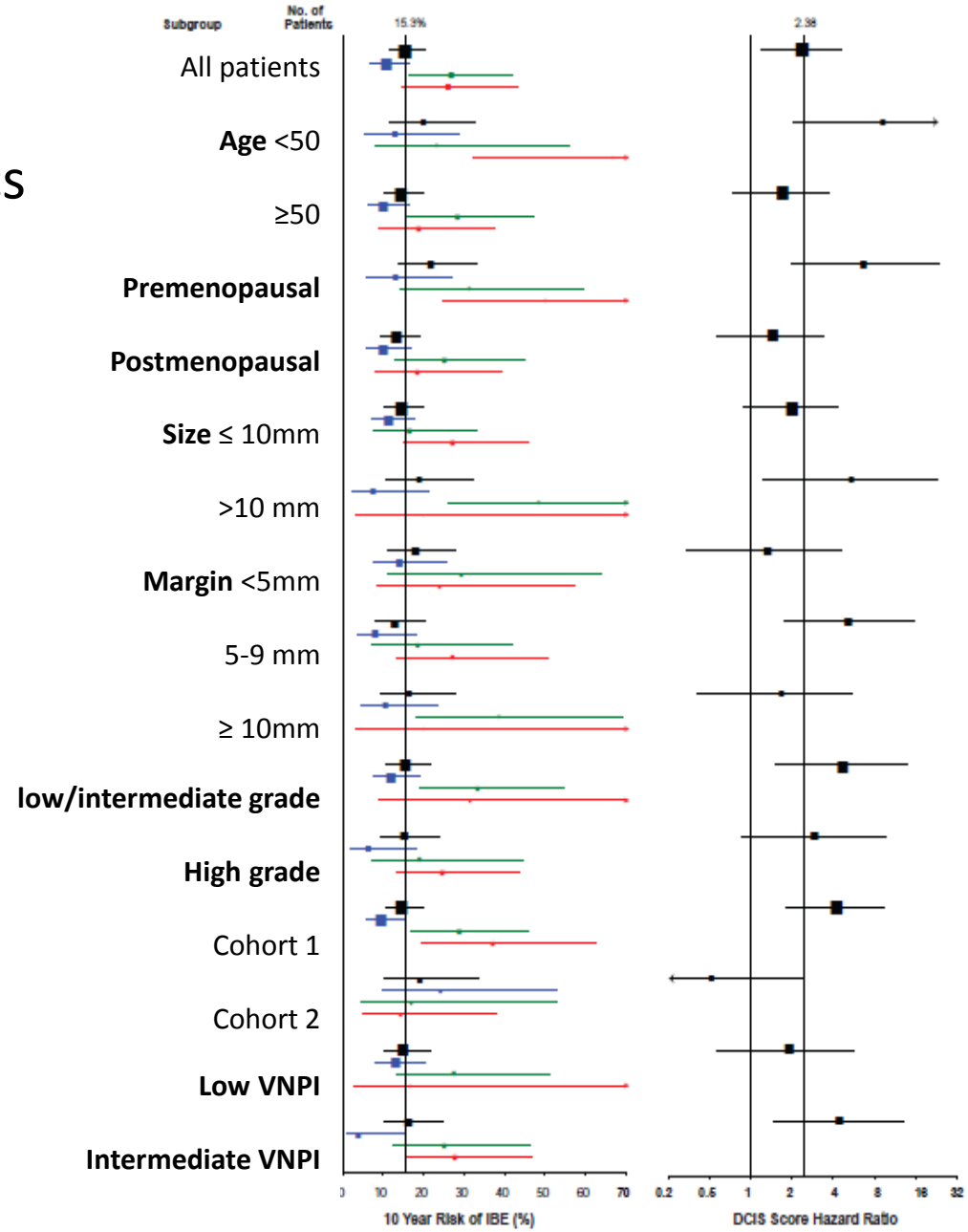
- Only factors with $p<0.05$ were included in multivariate analysis.
- Clinico-pathological factors showed no statistical significance included margin status, grade, comedo necrosis and Van Nuys prognostic index

DCIS score remains to be a significant factor in multivariate analysis demonstrates it provided additional information on recurrence risk beyond clinico-pathological factors

Solin LJ et al JNCI 2013

DCIS Score and Clinico-pathological characteristics

- For each clinico-pathological factors, a wide distribution of DCIS score values were observed within each subgroup
- Subgroup analysis generally showed the association of DCIS score with recurrence risk has similar trends and were directionally consistent with the overall group of patients



- Box size proportional to number of patient
  - overall
  - low risk group
  - intermediate risk group
  - high risk group

Solin LJ et al JNCI 2013

Summary

DCIS recurrence predictors

- traditional predictors – architecture, grade, size, necrosis, margin
- Molecular predictors – HER2, RB pathway (p16, COX2)
- Recurrence score
- Invasive recurrence more difficult to predict than in situ recurrence