versus 92.0% (HR 0.5; $p = 0.001$) for = 2 months, versus 96.1% (HR 0.3; $p = 0.029$) for =3 months. In multivariate Cox regression analysis timing =2 months was significantly related to an increased DMFS (HR 0.6). The 7-year disease-free survival was 82.3% for <2 months, versus 89.1% (HR 0.6; $p = 0.001$) for = 2 months, versus 90.2% (HR 0.5; $p = 0.066$) for =3 months. The 7-year disease specific survival (DSS) was 89.3% for <2 months, versus 94.4% (HR 0.5; $p = 0.009$) for =2 months, versus 97.1% (HR 0.4; $p = 0.148$) for =3 months. Also in multivariate Cox regression analysis timing =2 months was significantly related to an increased DSS.

Conclusion: Starting the radiotherapy in the second month or even the third month after lumpectomy seems to have a beneficial effect on distant metastasis and survival, and no effect on local control.

O-49. Accuracy of intraoperative radiology in assessing tumour proximity to resection margins during breast conserving surgery

Janes S, Singh SK, Isgar B. *New Cross Hospital, Wolverhampton*

The role of intraoperative radiology of the excised specimen during breast conserving surgery remains controversial. The aim of this study was to assess sensitivity, specificity and interobserver variation in close margin assessment using intraoperative radiology.

Radiological margins of 5, 10, 15 and 20 mm were compared to definitive histology in 116 consecutive patients. Sensitivity and specificity were calculated for each radiological measurement for the superior, inferior, lateral and medial specimen margins. Receiver-operator curves were plotted to determine the optimum area under the curve (AUC). A second observer measured margins in 43 patients, to assess interobserver variation.

A radiological standard of 15 mm maximised AUC for the inferior and lateral margins: AUC 0.81, 95% confidence interval (CI) 0.70–0.92, and AUC: 0.611, 95% CI 0.29–0.94 respectively. For the superior margin, using a 10 mm standard maximised the AUC: 0.725, 95% CI 0.52–0.97. For the medial margin, a 5 mm standard maximised the AUC: 0.79, 95% CI 0.60–0.98. Interobserver Kappa score for assessing close superior and inferior margins were 0.82 and 0.59 (indicating excellent and good agreement, respectively).

Different radiological margin measurements should be used for each margin to maximise the specificity and sensitivity of Intraoperative radiology during breast conserving surgery.

O-50. The expression of apoptosis-regulating proteins in usual ductal hyperplasia with known outcome

Shaaban AM, Jarvis C, Dodson A, Foster CS. *Leeds General Infirmary*

Members of the bcl-2 family are key regulators of apoptosis. Bcl-2 blocks apoptosis whereas Bax promotes apoptotic cell death. Their precise role in mammary carcinogenesis remains poorly understood. The relative expression of bcl-2 and bax would define the phenotypic behaviour of mammary epithelial cells. A case-control study was designed on 674 benign breast specimens received in three institutions in the period between 1979 and 1999. Study cases included all patients with benign breast lesions followed by in situ or invasive cancer of either breast at least 6 months after the benign lesion. Each study case was age and date of biopsy matched with three controls that had histories of benign breast lesions but did not develop breast cancer. Foci of hyperplasia of usual type (HUT) and adjacent morphologically normal lobules were identified from cases and controls and stained with monoclonal antibodies for bcl-2 and Bax. The results were correlated with ERα, ERβ and Ki67 expression. The median percentage of bcl-2 expression in HUT foci from patients who progressed to breast carcinoma was 50 whereas that of controls was 17.5, $P < 0.001$. A trend towards higher bcl-2 expression in normal lobules from patient who progressed to breast cancer was seen. Bax was highly expressed in normal lobules from controls when compared with cases ($P = 0.008$). HUT foci from cases exhibited significantly higher content of ERα, ERα/ERβ ratio and Ki67 when compared with controls. Using multiple logistic regression analysis, the correct classification rate of bcl-2 and Bax in classifying cases and controls was 70.2%. Our data show, for the first time, an early dysregulation of the levels of apoptosis-regulating proteins in normal and non-atypical hyperplastic foci of patients who progressed to breast cancer.

O-51. Cox-2 inhibition increases apoptosis in human ductal carcinoma in situ (DCIS) of the breast in a xenograft model

Barnes NLP, Flint P, Clarke R, Bundred NJ. *University Hospital of S Manchester & Christie Hospital*

Cyclooxygenase-2 (COX-2) expression is a poor prognostic factor in invasive breast cancer and DCIS. To determine the effect of COX-2 inhibition on human DCIS we compared the effect of Celecoxib (a COX-2 inhibitor) with placebo, in a nude mouse xenograft model, using DCIS samples collected from women undergoing mastectomy (after ethical approval and informed consent). The DCIS was dissected into lx2x2mm sections and eight sections were implanted subcutaneously into female nude mice. After 14 days, two DCIS xenografts were harvested and treatment was started with either 0.15% Celecoxib or control. Following 14 days of treatment, the remaining xenografts were harvested. The DCIS was assessed by immunohistochemistry for Ki67 (a marker of cell proliferation), apoptosis (H&E morphology) and COX-2 protein expression.

Celecoxib treatment decreased COX-2 expression ($p = 0.0001$; see table) and increased apoptosis ($p = 0.005$). No changes in cell proliferation were seen.

	Ki67 (%)		Apoptosis (%)		COX-2 (%)	
Day	14	28	14	28	14	28
Median Control	5.5	2.7	1.5	1.7	42.3	43.4
[IQR]	[2.9–9.0]	[1.6–5.8]	[0.9–2.7]	[0.8–3.0]	[35.8–56.7]	[34.8–48.2]
Median Treated	4.7	4.3	1.4	2.4	45.3	26.3
[IQR]	[1.9–6.8]	[2.0–7.5]	[1.0–1.7]	[1.3–3.7]	[32.3–58.2]	[16.3–39.2]
P value		0.5		0.005		0.0001

[IQR] = Interquartile range

Celecoxib significantly decreases COX-2 protein expression and increases the rate of apoptosis in human DCIS and is a promising adjuvant therapeutic strategy for Ductal Carcinoma In Situ.

O-52. Activated c-Src in ductal carcinoma in situ correlates with high grade and HER2 expression

Wilson G, Barnes N, Knox F, Swindell R, Kawakatsu H, Dive C, Bundred N. *University Hospital of S Manchester & University of California USA*

Background: The non receptor tyrosine kinase c-Src is downstream of HER2 and activation of c-Src influences response to Herceptin, and tumour progression and metastasis.

Aim: To determine the expression of activated c-Src in pure DCIS and determine if activated c-Src correlates with HER2 expression and clinicopathological parameters in DCIS.

Method: Immunohistochemical expression of activated c-Src using Clone 28 monoclonal antibody was evaluated in 78 patients (median age 55 years, range 39–71 years) with "pure" DCIS and a median follow-up of 60 months (range 24–240). HER2, HER4, ER and Ki67 levels were evaluated by immunohistochemistry. A HER2/HER4 score ≥ 2 was considered positive.

Results: Forty-three (out of forty-seven) HER2 positive tumours expressed active c-Src ($p < 0.015$). Strong expression of activated c-Src was also associated with high tumour grade ($p < 0.0005$) in 78 DCIS examined, but not epithelial proliferation (measured by Ki67, $p = 0.450$), tumour size, ER status and HER4 expression.

Characteristic		% DCIS Activated c-Src	P value
HER2	score ≥ 2	91% (43/47)	0.015
HER4	score ≥ 2	88% (22/25)	0.549
ER	positive	67.6% (52/77)	0.499
Tumour grade			
Low		10.3% (8)	
Intermediate		32% (25)	
High		57.7% (45)	$P < 0.0005$

Conclusion: Activation of c-Src is seen in high grade DCIS lesions with HER2 expression. Interruption of c-Src signalling with small molecule inhibitors may be therapeutically useful.

O-53. Accuracy of mammography in predicting histological extent of DCIS

Chakrabarti J, Evans AJ, James J, Pinder SE, Ellis IO, Macmillan RD. *Nottingham City Hospital*

Mammographic (MMG) extent is the main determinant for offering wide local excision for DCIS. It is recognized that this does not always correlate accurately with histological (HIST) extent. The aim of this study was to define the degree of variance between MMG and HIST measurement of DCIS and analyze the factors predicting a significant discrepancy.

The HIST and MMG data for 174 cases of DCIS were reviewed. The MMG size was bigger than the HIST size in 97(55.7%) and there was >10 mm difference in 18 (10.3%) of cases. The HIST size was bigger than the MMG size in 69 (39.7%) of cases and >10 mm difference in 30(17.2%) of cases.

The association between a variance in MMG/HIST extent and various factors is shown in the table below.

	MMG > HIST		HIST > MMG	
	>10 mm	1–10 mm	1–10 mm	>10 mm
Mean MMG size in (mm)	26.6	14.6	12.6	19.1
% High Grade	61.1	59.5	66.7	70.0
% Dense MMG	5.9	15.6	18.4	13.3
% Involved Margins	16.7	17.9	23.7	80

The only statistically significant finding was that cases with a HIST > MMG variance >10 mm were more likely to require further surgery for involved margins. We were unable to find factors that pre-operatively identified these women.

The important findings of this study are that MMG undersizes DCIS in over a third of cases and there is no reliable method for identifying this group. Other methods of pre-operative imaging should be explored.

O-54. Factors predicting recurrence in DCIS after breast conserving surgery with clear margins

Wilson G, Remouche S, Barns N, Knox F, Swindell R, Bundred N. *University Hospital of S Manchester*

Background: Factors predicting recurrence following breast conserving surgery (BCS) with clear margins for Ductal Carcinoma In Situ (DCIS) are largely unknown.

Aim: To determine recurrence rates and predictors of recurrence risk in patients who underwent BCS with clear margins (>1 mm) for pure DCIS.

Method: We reviewed all patients who underwent BCS ($n = 305$) for pure DCIS and then excluded patients with involved margins ($n = 76$) to determine what factors predicted recurrence in patients with clear margins ($n = 229$). ER, HER2, and Ki67 were measured by immunohistochemistry. A HER2 score ≥ 2 was considered positive.

Results: Margin status was a highly significant recurrence predictor ($p \leq 0.001$). Overall recurrence was 13.1% (30/229) in patients with clear margins at 5 years and 31.6% (24/76) with involved margins. In the group of women with clear margins after BCS ($n = 229$), high epithelial proliferation (measured by Ki67, $p = 0.044$) and HER2 positivity ($p = 0.042$) were associated with increased recurrence. 75% of recurrent DCIS had a Ki67 $\geq 10\%$ compared to 50% in the non-recurrent group 39% of HER2 positive DCIS recurred at 5 years compared to 16% HER2 negative. Tumour grade, size and age at diagnosis did not predict recurrence.

Patient Characteristics BCS + clear margins		Non-recurrent $n = 199$	Recurrent $n = 30$	p value (Log Rank)
HER2 positive (% ≥ 2)		44	70	0.042
Ki67	Median	10.1	13.2	
	Range	0.8–38	2.5–38.8	0.044
ER positive %		77.6	61.5	0.316
Tumour Grade	Low	16	1	
	Intermediate	47	8	
	High	118	21	0.496