adjuvant systemic therapy have been withdrawn from this analysis.
Analyses: Breast cancer specific survival (BCS) by life table.
Results: Median follow-up 8.8 years (2.5–13.9). 2,499 cases were ⩽ did not receive adjuvant therapy.
Table I, of these 2499 cases 1051 were <10 mm

Diam		n	BCS survival 10 yr %
<10 mm	All	1051	94±1
<10 mm	LN neg, gr I	569	97±1
<10 mm	LN neg, gr III	142	83±3

To base selection of cancers with over 90% survival without adjuvant therapy on ⩽10 mm, LN neg, is insufficient; grade must be considered.
Table II, 2499 <20 mm analysed by the Nottingham Prognostic Index (NPI) NPI = Grade (I-III) + LN status (Neg, + 1–3, + >4) +size (cm ×0.2)

NPI	NPI Group	n	BCS Survival 10 yr %
	All <20 mm	2499	86±1
<2.4	Excellent (EPG)	1117	96±1
2.41–3.4	Good (GPG)	886	92±1
3.41+	Other Groups	496	All <90%

The addition of LVI to NPI made no significant difference to the results.
Conclusion: The highest sensitivity and specificity for selection of tumours with BCS over 90% at 10 years is by NPI, selecting 80% of all tumours ⩽20 mm (n = 2,003) and additionally recognising 264 >20 mm. This compares with ⩽10 mm, LN neg, grade I selecting only 1,051 cases.

O-47 Gene expression profile associated with docetaxel resistance in breast cancer cells

I. Brown*, S.D. Heys, A.C. Schofield. *University of Aberdeen, UK*

The mechanisms of resistance to docetaxel are poorly understood. The purpose of this study was to investigate the genetic pathways involved in docetaxel resistance using a unique model of docetaxel resistance, which we have developed in breast cancer cells.
We made two breast cancer cell lines, MCF-7 and MDA-MB-231, resistant by exposure to increasing docetaxel concentrations. The resultant sublines were able to withstand 1, 10 and 30 μM of docetaxel. Alterations of gene expression were determined using Affymetrix Genechip cDNA microarrays, and subsequently validated by RT-PCR and western analysis.
After selecting out gene changes that were common between both sets of sensitive cell lines and their resistant sublines (>2 fold), further normalisation and statistical filtering (ANOVA, assuming unequal variances, and the Benjamin-Hobbson false discovery rate applied as a multiple correction factor with a significance level of $p < 0.01$), we identified a 14 probe-set, encoding 10 genes (including p-glycoprotein), which were significantly associated with resistance to docetaxel. This probe set was interrogated for predictive value using Support Vector Machine algorithm (using Fisher's Exact test and Gaussian kernal function) and Principal Component Analysis on Conditions was applied to identify similar groups of gene expression between all the cell lines.
These changes, therefore, may represent common mechanisms of resistance in breast cancer cells, and may be able to predict response. In addition, this is the first description, using microarray analysis, to identify the genetic pathways involved in the evolution of acquired resistance to docetaxel in a cell line model.

O-48 Tolerability of zoledronic acid – first safety data from the AZURE Trial (BIG01/04)

R.E. Coleman, H. Thorpe*, D. Cameron, R. Bell, D. Dodwell, M. Keane, M. Gil, J. Cousins, R. Burkinshaw, on behalf of AZURE investigators. *Weston Park Hospital, Sheffield, UK*

The AZURE trial was designed to determine whether Zoledronic acid (Z) improves the disease-free and bone metastasis-free survival of women with stage II/III breast cancer. 3207 eligible patients received (neo)adjuvant chemotherapy (CT) and were randomised to no additional treatment or Z 4 mg iv every 3–4 weeks during CT, then every 3–6 months to 5 years. To correspond with timing of CT, serious (SAE) and non-serious adverse event (AE) data within 6 months of randomisation were compared.
939 SAE and 33859 AE have been reported to date. No significant differences in the numbers of patients with any SAE (324 [20%] CT, 373 [23%] CT+Z), or neutropaenic sepsis SAE (157 CT v 155 CT+Z respectively) were seen. CTC grade 3/4 AE occurred in 4.6% and 4.8% with CT and CT+Z respectively. The frequency of CT dose reductions (17% CT, 14% CT+Z) and median duration of CT (3.52 months CT, 3.48 months CT+Z) were similar, confirming that Z has no significant effect on CT delivery. 9 cases of osteonecrosis of the jaw have been confirmed to date (all reported cases).
This is the largest safety analysis of Z in patients without the confounding influence of metastatic disease and indicates that Z can be safely combined with adjuvant chemotherapy.

O-49 A phase III trial of Gemcitabine plus Docetaxel (GD) versus Capecitabine plus Docetaxel (CD) for patients (pt) with anthracycline-pretreated metastatic breast cancer

S. Chan*, R. Sharma, G. Romieu, T. Huober, T. Delozier, M. Tubiana-Hulin, A. Schneeweiss, A. Lluch, A. Llombart, A. du Bois, E. Carrasco, A. Thareau, P. Fumoleau, on behalf of trial investigators. *Nottingham City Hospital, UK*

Background: Patients (Pts) with anthracyclines pre-treated MBC frequently receive combination chemotherapy with a taxane and an antimetabolite such as gemcitabine or capecitabine. This trial compared the-(GD) combination with the (CD) combination, in this clinical setting. The primary objective of the trial was a comparison of the progression-free survival (PFS) difference between the two treatment groups, and the secondary objectives included overall response rate (ORR), time to treatment failure (TtTF), overall survival (OS), and toxicity assessments. In a previous analysis GD demonstrated similar efficacy to CD but with a better non-hematological toxicity profile [Chan et al, ASCO 2005]. This reports the final analysis of the results including OS.
Methodology: Pts with histologically/cytologically confirmed MBC, who had received an anthracycline-based regimen in the neoadjuvant/adjuvant/or first-line metastatic setting, were randomized to GD ($G = 1000\ mg/m^2$ d1, 8; $D = 75\ mg/m^2$ d1) or CD ($C = 2500\ mg/m^2$ daily d1 to 14; $D = 75\ mg/m^2$ d1) q21 days.
Results: Characteristics of the 305 included patients (GD = 153; CD = 152) were previously reported. A median of 6 cycles was delivered on both arms. CTC grade 3/4 hematologic toxicity was similar in both arms, except for grade 3/4 thrombocytopenia GD = 11%; CD = 3%; $p = 0.014$). The fact that blood test was performed at day 8 in the GD (pts received iv chemotherapy) but not the CD arm of the trial, may explain this difference. Nonhematologic toxicities were low in both arms, but

significantly different: grade 3/4 toxicities were more pronounced in CD arm; diarrhea (GD = 8%; CD = 18%; p = 0.0088), mucositis (GD = 4%; CD = 15%; p = 0.0008), and hand-foot syndrome (GD = 0%; CD = 26%; p < 0.0001). 13% of pts stopped therapy due to adverse event on GD vs 30% on CD. Best overall response rates in both arms were 32% (p = 0.93). Median PFS (1% of patients censored in GD and 7% in CD) was 8.05 months (95% CI 6.60–8.71) in GD arm, and 7.98 (95% CI 6.93–8.77) in CD arm (log-rank p = 0.121). Of notice when interpreting this data, 11% in GD arm vs 26% in CD arm received additional chemotherapy before progression. With a median follow-up of 19.2 months, and 23% of patients censored, the median overall survival was 19.29 months (95% CI 15.57–23.59) on GD arm, and 21. 45 (95% CI 17.12–24.94) on CD arm (log-rank p = 0.982).
Conclusion: These data suggest that the two regimens are comparable in efficacy; however a more favorable toxicity profile on GD arm may be a determining factor in selecting a better treatment option. Exploratory sub-group efficacy analysis results will be presented at the meeting.

O-50 Role of Caveolin-1 expression in docetaxel resistance in breast cancer cells

J.N. Sangrithi-Wallace*, I. Brown, S.D. Heys, A.C. Schofield. *University of Aberdeen, UK*

Docetaxel is very effective in the treatment of breast cancer. However, despite its efficacy, resistance to docetaxel remains a significant problem and the genetic pathways involved in docetaxel resistance are not well understood. We have previously used comparative genomic hybridization (CGH) and bacterial artificial chromosome (BAC) fine-mapping on docetaxel resistant breast cancer cell lines to identify and accurately map the minimal chromosomal regions that are modified in docetaxel-resistant cells relative to their parental docetaxel-sensitive cells. Caveolin-1 is one of the candidate genes identified by CGH and BAC-mapping to be amplified in docetaxel-resistant MDA-MB-231 cells. The role of caveolin-1 in docetaxel resistance was investigated by modulating its expression in these cells.
Caveolin-1 protein expression in docetaxel-resistant cells relative to parental, docetaxel-sensitive cells was analysed by western blot analysis. Caveolin-1 siRNA or scrambled control siRNA was transfected into docetaxel-resistant cells and mRNA knockdown was monitored by RT-PCR. MTT cytotoxicity assay was used to monitor effects on docetaxel resistance in the transfected cells.
Caveolin-1 protein expression in docetaxel-resistant cells was found to be increased 5.96-fold ($\pm$1.63) relative to docetaxel-sensitive cells. Complete knockdown of caveolin-1 mRNA expression was achieved in docetaxel-resistant cells transfected with caveolin-1 siRNA compared to scrambled control siRNA, 48 hours post-transfection. Pilot data from MTT assays demonstrated an increase in sensitivity in caveolin-1 siRNA transfected cells compared with control siRNA transfected cells, at lower concentrations of docetaxel
This preliminary study highlights that overexpression of caveolin-1 may be involved in resistance to lower concentrations of docetaxel in MDA-MB-231 breast cancer cells.

O-51 Expression of thioredoxin system proteins in locally advanced breast cancer – correlations with response to anthracycline based chemotherapy (C/T).

L. Zhang*, S. Chan, A. Mukherjee, M. Shehata, K. Huber, I. Ellis, P. Patel, S. Martin. *University of Nottingham and Nottingham City Hospital, UK*

The thioredoxin (Trx) system helps maintain a reducing environment in cells and regulates many key biologic processes including cellular growth, transcription factor activity, acting as an antioxidant and regulator of apoptosis. Expression of thioredoxin binding protein (TXNIP), a negative regulator of Trx, is frequently lost in tumor tissues and cell lines. Thioredoxin over expression is associated with resistance to several chemotherapeutic agents *in vitro*.
The present study examined, in 60 locally advanced breast cancer patients treated by FEC/FAC for 4–6 cycles in the neoadjuvant setting, whether the expression of Trx and related proteins (TXNIP and thioredoxin reductase, TrxR) were associated with resistance to C/T. Standard immunohistochemical techniques were used to assess protein expression both pre- and post C/T and results correlated with clinical response.
Tumours with high pre-C/T Trx and TrxR expression showed a lower complete response rate (CRR) than those with low expression ($P < 0.05$). High expression of TXNIP also correlated with a higher CRR. Trx expression significantly increased after anthracycline therapy (24.5% increase; $P < 0.001$). There was no significant increased expression of either TrxR or TXNIP following C/T. Tumors with high Trx expression showed significantly lower TXNIP expression and vice versa. We conclude that Trx and TXNIP may be clinically useful biomarkers for predicting response to anthracycline based C/T and that if using Trx expression to monitor response then TXNIP should also be assessed.

O-52 The basal phenotype (BP) is highly expressed in locally advanced breast cancer (LAPC) but does not predict response to neo-adjuvant anthracycline based chemotherapy

A. Mukherjee*, M.A. Shehata, R. Sharma, A.S. Dhadda, K. Huber, C. Paish, I. Ellis, S. Chan. *Nottingham City Hospital, UK*

Background: The prognostic value of BP is well established. This study aimed to investigate the incidence of BP in LAPC and evaluate it as a marker for predicting response to neo-adjuvant anthracycline based chemotherapy.
Methodology: The study involved 60 LAPC patients at Nottingham City Hospital treated with 6 cycles of FEC/FAC neo-adjuvant chemotherapy between December 1996 and January 2007. A pragmatic definition of BP as immunophenotypic evidence of basal cytokeratins CK5/6 and/or CK14 expression was used. Standard immunohistochemical techniques were employed to assess marker expression in pre-chemotherapy core biopsies and results correlated with clinical response (RECIST).
Results: Of 60 cores stained, 34 (56%) were BP positive. 20 of them were also ER negative (33% overall). The BP positive subgroup (34/60) responded as follows: 30 responders (88%) (15 CR and 15 PR) versus 4 non-responders (12%) (3 SD and 1 PD). In the BP negative subgroup (26/60), there were 22 responders (84%) (10 CR and 12 PR) versus 4 non-responders (16%) (3 SD and 1 PD). 14 of the 60 (23%) patients suffered recurrences: 10 (71%) were BP positive while 4 (29%) were negative. Of other response predictors such as Her2/Topo2α, Ki67 and p53, only Ki67 negativity predicted resistance in this cohort (p = 0.007).