Sixteen (trial 0020) and four (trial 0021) patients provided blood samples at intervals over 28 days for full PK profiles after the first injection. Thereafter monthly samples were obtained from 27 patients (trial 0020) and from all patients in trial 0021 (n = 194), and continued for up to 30 months (i.e., 30 injections) after the first dose.

The shape of the observed plasma FAS profiles following i.m. injection of the LA formulation was very similar in both studies. Gmean trough plasma concentrations increased slightly after the first injection from 2.4 ng/ml and 2.6 ng/ml, increasing to 6.5 ng/ml and 6.2 ng/ml in trials 20 and 21 respectively, reaching steady state after approximately three to six doses. Steady-state plasma FAS exposures (AUC_{ss}), was reached in 3–6 doses and predicted according to a structural model were very similar for both studies (trial 0020; 336 ng.d/ml, trial 0021; 294 ng.d/ml), and, by comparison with single dose data, represents approximately two-fold accumulation. The repeated i.m. injections were well tolerated in both studies. The results of this study show that 5 ml and 2.5 ml injections of FAS 250 mg are equally effective in maintaining plasma FAS levels in the therapeutic range for at least 30 months, and support the use of either dosing regimen in the clinical setting.

O-24. ICI 182,780 ('FASLODEX'™) IS AT LEAST AS EFFECTIVE AS ANATROZOLE (ARIMIDEX™) IN POST-MENOPAUSAL WOMEN WITH ADVANCED BREAST CANCER PROGRESSING ON PRIOR ENDOCRINE TREATMENT

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ICI 182,780 ('Faslodex'™) (FAS) is the first of a new class of anti-oestrogen: an Estrogen Receptor Downregulator. We report the results of two, randomised, multicentre, parallel-group trials, (0020 European and 0021 North American) comparing the efficacy and tolerability of a monthly intramuscular injection of 250 mg FAS with that of a daily oral administration of anastrozole (Arimidex™) (AN) 1 mg in post-menopausal (PM) women with advanced breast cancer (ABC) progressing on prior endocrine treatment. Primary endpoint was time to progression (TTP). Secondary endpoints included objective response (OR) rate, duration of response (DOR), and tolerability. Patients were recruited from 1997 to 1999 and randomly assigned to either FAS or AN. The median follow-up duration was 14.4 months (mo) for study 0020 and 16.8 months for study 0021, with disease progression in 83% of randomised patients. Both drugs had similar tolerability profiles and were well tolerated. The incidence of withdrawals due to a drug-related adverse event was 0.9% for FAS and 1.2% for AN. Data from all major efficacy endpoints are shown in Table.

	0020		0021	
	FAS	AN	FAS	AN
Median TTP (months)	5.5	5.1	5.4	3.4
OR (CR + PR)	20.7%	15.7%	17.5%	17.5%
Clinical Benefit Rates	44.6%	45%	42.2	36.1
Median DOR	14.3	14.0	19.3	10.5

This is the first anti-oestrogen, which is at least as effective as the new generation aromatase inhibitor, AN in PM women with ABC and is especially noteworthy since most patients had had prior tamoxifen treatment. FAS will be a valuable additional treatment option for ABC in these patients.

O-25. CORRELATION OF IMMUNOHISTOCHEMISTRY (IHC) AND GENE MICROARRAY ANALYSIS OF BREAST BIOPSIES FROM A PREOPERATIVE ENDOCRINE THERAPY TRIAL

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Background: IHC is used to assign predictive/prognostic marker status in breast cancer, but is limited by antibody availability. Microarray analysis rapidly evaluates expression of large numbers of genes and is a potentially efficient screen for identifying novel markers. However, for this purpose microarray and IHC assignment of standard markers must correlate.

Methods: Breast biopsies were collected before and after preoperative endocrine therapy from patients in a phase III trial comparing letrozole with tamoxifen. RNA was extracted from non-dissected frozen biopsies and converted to cRNA. Estrogen receptor (ER) and trefoil factor 1 (PS2) expression was determined with the FL Affymetrix array. ER and PS2 IHC were performed using standard methodology. Protein expression was scored by the Allred method (intensity + proportion); a score > 3 was considered positive.

Results: Expression profiles determined by array or IHC were scored as present and absent and expression levels were also recorded. IHC and gene expression profiles were available for 29 biopsies (17 pre- and 12 post-treatment) from 24 patients. The presence or absence of ER determined by array was concordant with IHC scored by the Allred method in 100% (29/29) of biopsies, however levels of ER mRNA measured by array did not correlate with IHC protein levels ($r = 0.267$, $p = 0.161$). Presence or absence of PS2 determined by array and IHC was concordant in 75% (21/28, 1 assay failure) of biopsies. A significant correla-

tion (Pearson r-value 0.463, p = 0.013) between PS2 mRNA and protein expression levels existed.

Conclusions: The presence or absence of ER and PS2 was partially concordant between IHC and microarray analysis. These findings suggest Affymetrix analysis complements IHC and supports the use of this technology for the identification of novel predictive and prognostic markers for breast cancer.

O-26. CONCORDANT LOSS OF HETEROZYGOSITY IN TUMOUR PAIRS OF BREAST CANCER FAMILIES AS A PREDICTOR OF GERMLINE MUTATIONS

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Loss of Heterozygosity (LOH) can arise randomly in sporadic breast cancer due to genetic instability. However, concordant LOH in tumours from related individuals may point to a germline mutation. We looked for LOH at BRCA markers in tumour pairs of at-risk families in order to evaluate its predictive value in selecting those likely to harbour a mutation.

67 tumour pairs and 54 sporadic breast cancer control cases were studied. 5 microsatellite markers flanking and intragenic to each of the BRCA1 and BRCA2 genes, plus a control marker, were evaluated for LOH by analysis of fluorescent labelled PCR products in an automated sequencer.

In familial cancers, the LOH/Informative ratio at BRCA markers was higher than in sporadic controls (54.7% vs. 38.3%, p = 0.001), and higher than at the control marker (54.7% vs. 29.3%, p = 0.004).

7/17 informative families showed concordant homoallelic LOH for 2 or more markers at BRCA1 (O/E = 57.7, p < 0.001) and another 1/7 did so at BRCA2 (O/E = 31.1, p = 0.04). Mutational analysis is ongoing.

Preliminary results indicate that, in tumour pairs, concordant homoallelic LOH at two or more markers may predict the site of a predisposition gene.

O-27. HEAT SHOCK PROTEIN 27 AS A MARKER OF MALIGNANT POTENTIAL IN NORMAL, BENIGN AND INVASIVE BREAST LESIONS

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Heat shock proteins (hsps) occupy a central role in the regulation of intracellular homeostasis. Previously it has been shown that hsp27 is overexpressed in breast cancer. We have investigated its expression in normal and hyperplastic breast lesions that carry a mild increased risk of developing breast cancer. Forty-nine biopsy specimens of normal breast, 47 hyperplasia of usual type (HUT) and 125 primary breast cancers were included in this study. Staining for hsp27 was performed using heat treatment antigen retrieval and a murine monoclonal antibody (Novocastra Laboratories Ltd.). Positive staining was cytoplasmic and quantified by measuring the mean optical density (OD) for each case using a morphometric image analysis system. In addition the % of positive stained cells was estimated manually.

A progressive increase in hsp27 expression was seen from normal through HUT to invasive cancer. In normal breast, the % expression was significantly lower than the expression in HUT and cancers (p < 0.001). The mean % positivity was 6% for normal breast, 23.5% for HUT and 54.9% for invasive tumours. The mean OD was 0.32 for normal breast, 0.49 for HUT (p < 0.001) and 0.57 for cancers (p = 0.01).

Our data show a previously undescribed increase in the expression of hsp27 from normal through precancerous breast to malignancy. This may be an important early event in mammary carcinogenesis.

O-28. DELAY TO FIXATION OF INVASIVE BREAST CARCINOMA: EFFECT ON MITOTIC COUNT, MIB1, ER AND P53 EXPRESSION

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Several small studies have suggested that a delay prior to fixation has an effect on the mitotic count of a tumour. This is an essential component of histological grade of invasive breast cancer and grade itself, as part of the Nottingham Prognostic Index, plays an important role in treatment selection. We have examined the effect of a delay to fixation in 4% Baker's formal calcium of 30, 60 and 120 minutes in a series of 25 breast cancers.

Tumours were received fresh and multiple small portions (approximately 0.5 mm^3) from the periphery sampled. One was immediately placed in formalin; the remainder were placed into fixative after delays of 30, 60 and 120 minutes. After routine processing, H&E stained sections were examined using strictly defined criteria for mitoses by one observer (NB). Sections were also assessed for MIB1, ER and p53 expression.

A significant decrease in mitotic count was seen with a 1 hour delay in fixation (p = 0.016). The mitotic score (1–3) ascribed to the mitotic count and histological grade also tended to be lower with delayed fixation. No significant decrease in MIB1 labelling (p = 0.808) or ER expression (p = 0.079) was seen. A decrease in intensity and percent nuclear staining with p53 was seen after a 2-hour delay in fixation.

A delay in fixation of an only a relatively short time influences mitotic count, with the potential for alteration of histological grade. This effect may be important, not only for fresh samples not immediately placed into fixative but also for tumours within large fatty specimens (for example mastectomy samples) not rapidly penetrated by fixative.