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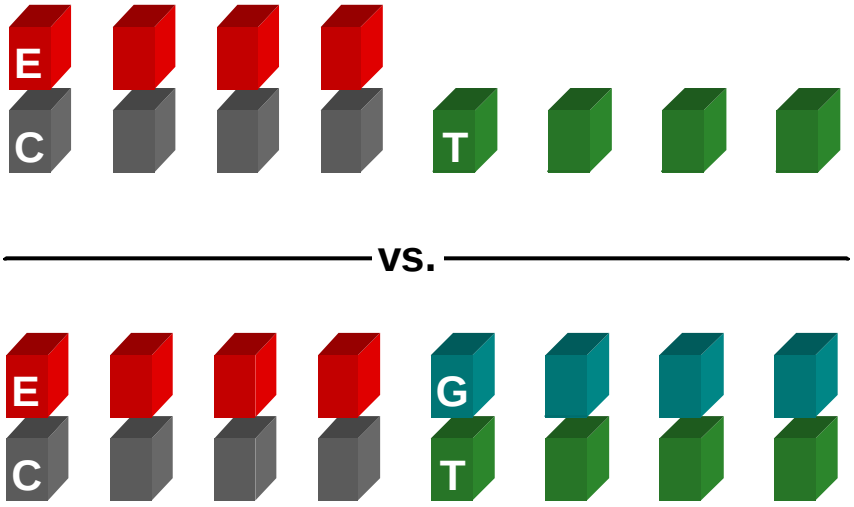
tAnGo

Paclitaxel, Anthracycline, Gemcitabine & Cyclophosphamide



A randomised phase III trial of gemcitabine in paclitaxel-containing, epirubicin-based, adjuvant chemotherapy for women with early stage breast cancer

CANCER RESEARCH UK

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1. Trial Summary

Title: '*tAnGo*', a randomised phase III trial of gemcitabine in paclitaxel-containing, epirubicin-based, adjuvant chemotherapy for women with early stage breast cancer

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Number of subjects to be enrolled:	3000
Randomisation:	1:1
Phase:	III
Indication:	Early stage, higher risk female breast cancer

1.2 Rationale

Despite the therapeutic advances of the last few years, many women still succumb to the complications of metastatic breast cancer and the development of more effective adjuvant chemotherapy remains a priority in improving the treatment of this disease. The relatively modest impact of conventional adjuvant chemotherapy has been confirmed as a 24% global reduction in the risk of relapse or death (i.e. hazard ratio (HR) = 0.76) and a 15% reduction in the risk of death (i.e. HR = 0.85) in an 18,000 patient meta-analysis of 47 trials by the Early Breast Cancer Trialists' Collaborative Group (Oxford Overview). The incorporation of anthracyclines into combination regimens provides additional benefits, with a HR of 0.88 estimated by the 1998 Oxford Overview, in relation to CMF [1], and a HR $\leq$ 0.7 reported for trials with epirubicin-based adjuvant regimens (NCIC-MA5, *NEAT*, Mc*NEAT*).

More recently, the 3000-patient CALGB 9344 and NSABP B28 trials have shown that the sequential addition of four cycles of paclitaxel to standard therapy with four cycles of doxorubicin and cyclophosphamide may further reduce the risk of recurrence. The latest 1054 event intent-to-treat-analysis (69 months median follow-up) of CALGB 9344 reported that the risk of relapse or death was reduced by 17% (HR = 0.83, 95% C.I. 0.73-0.94, p=0.0013) and the risk of death by 18% (HR = 0.82, 95% C.I. 0.71-0.95, p=0.0061) [2]; for NSABP B28 the respective figures are 17% (disease-free survival HR = 0.83, 95% C.I. 0.73-0.95, p=0.008) and 6% (overall survival HR = 0.94, 95% C.I. 0.78-1.12, p=0.46) [3].

Based on pre-clinical evidence of a potentially favourable interaction between paclitaxel and gemcitabine, as well as encouraging activity in advanced breast cancer, with a particularly high complete response rate in heavily pre-treated patients, it seems plausible that the addition of gemcitabine to paclitaxel in the AC-T regimen may further improve the disease-free and overall survival of patients with early stage disease. The favourable results of a recent pivotal randomised phase III trial comparing paclitaxel and gemcitabine in combination with single agent paclitaxel in patients with anthracycline pre-treated metastatic disease lend weight to this hypothesis [4].

1.3 Primary objective

To determine whether EC-TG (epirubicin 90 mg/m^2 and cyclophosphamide 600 mg/m^2, day 1, q 3 weeks; followed by paclitaxel 175 mg/m^2/3 hr infusion, day 1 and gemcitabine 1250 mg/m^2 days 1 and 8, q 3 weeks) improves disease-free survival compared with EC-T, in women presenting with higher risk, early stage breast cancer.

Primary endpoint
- 5-year disease-free survival

Secondary endpoints
- 5- and 10-year overall survival comparisons
- 10-year disease-free survival
- Toxicity, dose-intensity and tolerability
- Serious adverse events

Study design and methodology
- Randomised, phase III, multi-centre, prospective, randomised trial, accruing 3000 patients over 3-4 years.

Sample size determination
- We estimate the 5-year disease-free survival rate for patients treated with EC-paclitaxel in this higher risk group will be approximately 70%. The accrual of 1500 patients into each treatment arm will allow absolute differences in survival in excess of 5% to be detected at the 5% (2-sided) level of significance with an 85% power, in excess of 7% with a 99% power, and in excess of 10% with a 99.9% power.

Analysis
- Primary statistical analysis will be based on the comparison of the curves of cumulative disease-free survival (estimated by the Kaplan-Meier method) using the log-rank test with a minimum of 18 months (i.e. approximately 280 events), 24 months (i.e. approximately 550 events) and 60 months (i.e. approximately 920 events) follow-up on an intention-to-treat basis.
- Exploratory sub-group analyses will be facilitated by stratification groups (see section 13 for details).

First 130 patient detailed safety study
- To provide a sensitive measure of any treatment related clinical or sub-clinical radiological abnormalities, pulmonary function impairment and gas diffusion deficit and reduction in left ventricular ejection fraction in the first 130 patients randomised. All adverse events will be included in the analysis which will take place after all 130 patients have completed adjuvant chemotherapy and radiotherapy.
- Assuming a 1.5-2% incidence of symptomatic pulmonary toxicity ordinarily attributable to gemcitabine, the accrual of 65 patients into each treatment arm would detect a 10-fold difference in the risk (i.e. observed vs. expected) of symptomatic pulmonary toxicity relative to this, with an 80% power at the 5% level of significance (2-sided).

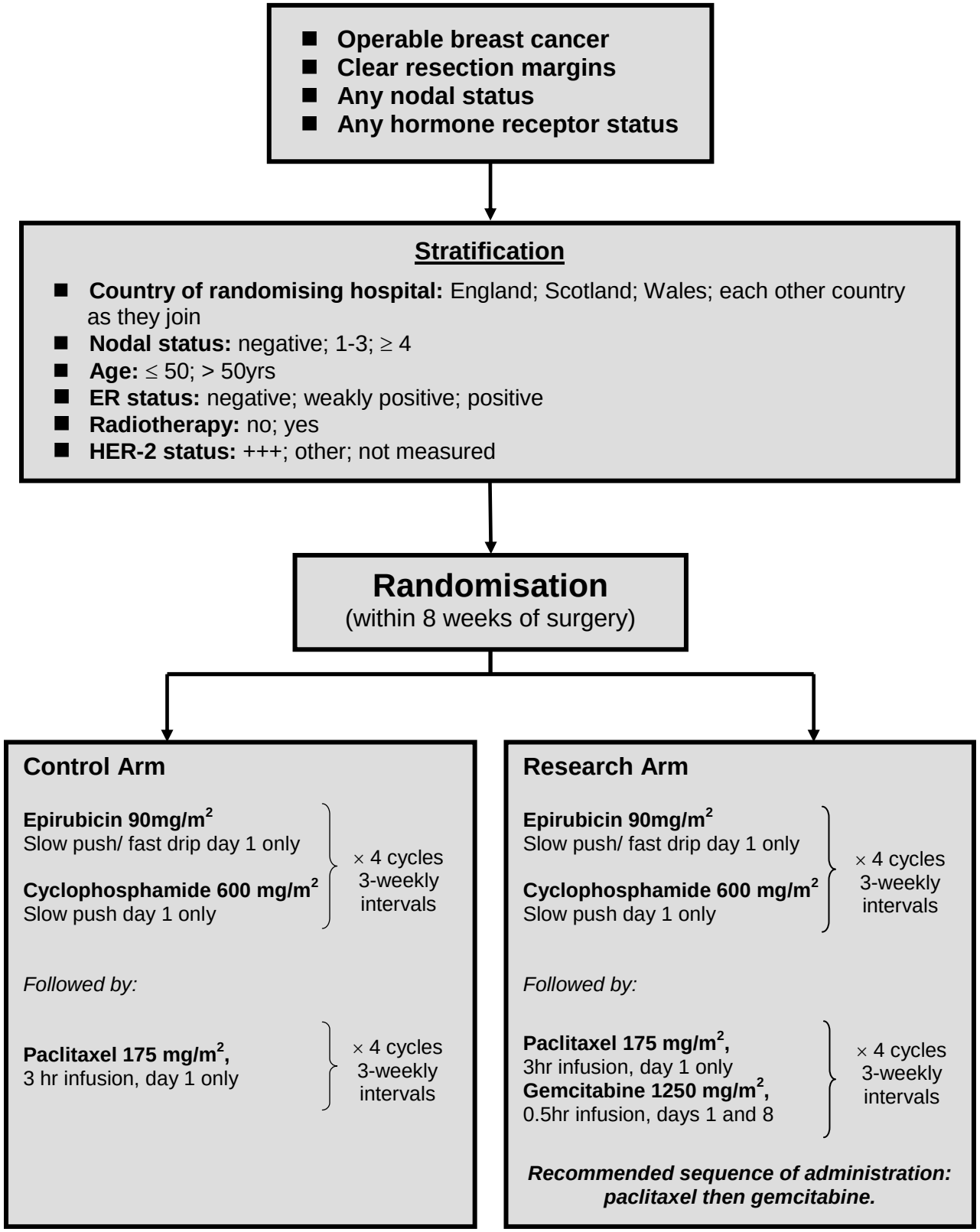
Quality of Life sub-study
- An optional sub-study addressing quality of life will be assessed in a cohort of 500 patients.

Duration of treatment
- Equal in both arms, 24 weeks (approx. 6 months)

Key inclusion criteria
- Women with completely excised, newly diagnosed, early stage breast cancer
- Definite indication for adjuvant chemotherapy
- Any nodal status
- Any hormone receptor status
- Adequate marrow, hepatic and renal function
- 18 years or older, with an ECOG performance status of 0, 1, or 2

Key exclusion criteria
- Prior chemotherapy
- Prior radiotherapy
- Evidence of metastatic spread
- Pregnancy or lactation

2. Trial Schema



For recommended anti-emetic medication and prophylaxis for paclitaxel-related acute hypersensitivity reactions, please refer to section 7.4.

3. Hypothesis

In women with early stage breast cancer, the addition of gemcitabine to paclitaxel-containing, epirubicin-based, adjuvant chemotherapy provides significantly superior disease-free and overall survival, without excess toxicity or prolonged adverse impact on quality of life.

4. Introduction

Breast cancer is the most common malignancy to afflict women in the Western World, with approximately 40,000 new cases each year in the UK alone, and an annual mortality of almost 13,000 (see Table 1). One woman in 11 is affected at some time in her life. Whilst the age incidence curve for breast cancer is similar to most other solid tumours, it remains the major killer of women in child bearing years. Besides the morbidity and mortality caused to the woman herself, breast cancer can also have profound psychological and economic consequences for the family. More effective treatments are urgently required. Our knowledge of the natural history of this disease suggests that any significant improvement in outcome will depend upon the development of more effective adjuvant therapy for women presenting with early stage disease [6]. However, the introduction of newer, more expensive agents may well present a major challenge to NHS resources [7-9], a burden which might well necessitate more precise quantification of individual risk as well as definition of those factors, both clinical and molecular, which individually or in combination predict treatment benefit.

Table 1: **Female breast cancer incidence (a) and mortality (b) in the UK, ranked against other cancers affecting women [5].**

(a) Incidence (1999)

Breast	40,990 (30%)
Large bowel (colorectal)	16,810 (13%)
Lung	14,740 (11%)
Ovary	6,800 (5%)
Body of uterus	5,200 (4%)
Non-Hodgkin's lymphoma	4,300 (3%)
Pancreas	3,670 (3%)
Bladder	3,640 (3%)
Stomach	3,480 (3%)
Malignant melanoma	3,430 (3%)
Other	33,110 (22%)
Females: all malignant neoplasms excluding NMSC	136,160 (100%)

(b) Mortality (2000)

Lung	13,040 (18%)
Breast	12,990 (17%)
Large bowel (colorectal)	7,630 (10%)
Ovary	4,660 (6%)
Pancreas	3,540 (5%)
Oesophagus	2,550 (3%)
Stomach	2,530 (3%)
Non-Hodgkin's lymphoma	2,220 (3%)
Leukaemia	1,860 (2%)
Bladder	1,680 (2%)
Other	21,270 (29%)
Females: all malignant neoplasms	74,420 (100%)

5. Background and rationale

5.1 Adjuvant therapy for early stage breast cancer

Early stage breast cancer is by definition grossly limited to the breast and ipsilateral axillary nodes and amenable to surgical resection. The development of occult systemic (and local) micrometastatic deposits may predate diagnosis and later progress to a clinically detectable recurrence, and eventually prove fatal. Twenty-year follow-up studies of women presenting with early stage breast cancer in the UK and North America in the 1940's established the limitations of purely locoregional treatment in modifying the natural history of early stage disease [10,11]. Together with pre-clinical work, which defined susceptibility of micrometastases to chemotherapy [12], these studies provided the justification for the clinical development of systemic adjuvant chemotherapy in the 1970's [13].

Thirty years on, there is now an incontrovertible body of evidence to demonstrate the success of this endeavour. In an overview of 47 trials of prolonged polychemotherapy versus no chemotherapy involving 18,000 women, the Early Breast Cancer Trialists' Collaborative Group (EBCTCG) analyses have confirmed that adjuvant chemotherapy reduces the annual odds of recurrence by 24%, and the odds of death by 15% [1]. This reduction in recurrence emerges chiefly during the first 5 years of follow-up, whereas the survival advantage grows throughout the first 10 years. Subgroup analyses of these data have provided further information about relative benefit from treatment, by axillary lymph node involvement, age, menopausal status and oestrogen receptor (ER) status. These are now discussed.

5.2 The 1998 Oxford Overview sub-group analyses

Nodal status

The proportional reduction in risk of recurrence afforded by chemotherapy is similar for women with node-negative and node-positive disease. For women under 50 years, the 10-year survival of those with node-negative disease is increased from 71% to 78% (an absolute benefit of 7%) whilst the 10-year survival of those with node-positive disease increases from 42% to 53% (an absolute benefit of 11%).

Age

For women aged 50-69 years, the proportional reduction in mortality is smaller than that observed in younger women. In the older age group, the 10-year survival of node-negative women increases from 67% to 69% with polychemotherapy and for those with node-positive disease, the 10-year survival increases from 46% to 49%.

Tumour ER status

Amongst women aged under 50, the Overview shows substantially reduced risk of recurrence with combination chemotherapy, both for those with ER-poor disease (40% [SD 7]) and those with ER-positive tumours (33% [SD 8]). These figures are not significantly different from one another. By contrast, among women aged 50-69, the proportional reduction in recurrence appeared to be nearly twice as large in women with ER-poor disease (30% [SD 5]) as in those with ER-positive tumours (18% [SD 4]), and the difference between these effects is conventionally significant (heterogeneity between proportional reductions $\chi 21=4{\cdot}5$; $2p=0{\cdot}03$).

The effects of polychemotherapy on recurrence also appeared to be somewhat smaller for women with ER-positive disease when the two age groups are combined (heterogeneity, stratified for age, $\chi 21=4{\cdot}9$; $2p=0{\cdot}03$). However, in both age ranges, the reduction in recurrence among women with ER-positive disease is highly significant (both $2p<0{\cdot}00001$), indicating that in neither age range can such hormone-receptor measurements discriminate a group of women who would fail to benefit from treatment. The pattern of somewhat smaller proportional risk reductions among women with ER-positive tumours was also seen for mortality, although the heterogeneity of this effect was not significant.

Treatment comparisons: (a) duration

Data are available from 11 randomised trials, involving over 6000 patients, which address the optimal treatment duration question. Most involve CMF-based adjuvant therapy. These studies comprise comparisons of longer regimens (employing at least 6 months of the same polychemotherapy) versus shorter regimens (of less than 6 months). Overall, there is a border-line significant 7% (SD 4) reduction in recurrence with longer therapy ($2p=0{\cdot}06$), but no improvement in survival (1% [SD 5] increase; ns), either globally, or amongst women aged under 50. These trials involved 6104 women with a total of 2677 recurrences, and 2076 deaths.

Treatment comparisons: (b) anthracyclines

The Oxford Overview identified 11 randomised trials, involving a total of nearly 7000 patients, which compare anthracycline-containing regimens, such as FAC or FEC, versus CMF alone. Taken together, the addition of anthracyclines yielded a further 12% (SD 4) proportional reduction ($2p=0{\cdot}006$) in the odds of recurrence, with no significant heterogeneity between the effects seen in the different trials. The EBCTCG also reports a marginally significant 11% (SD 5) proportional reduction in mortality with the anthracycline-containing regimens ($2p=0{\cdot}02$). The log-rank p value of 0·02 for mortality indicates that the absolute extra benefit could be anywhere from about zero to about double the 2·7% (SD 1·4) difference observed at 5 years. About 70% of these women were aged under 50 and their overall results were similar.

The "2000" Oxford Overview

Publication is eagerly awaited; however, informal presentation of these data in 2000 were largely confirmatory of the 1998 publication, albeit providing greater statistical security. For obvious reasons they cannot be cited in detail at this stage.

5.3 Epirubicin in adjuvant therapy

Two more recent randomised trials have been instrumental in securing the approval of epirubicin for use in the adjuvant therapy of women with node-positive, early stage breast cancer in the USA. Data from neither were available for incorporation in the 1998 Oxford Overview.

CAN-NCIC-MA-5

This study randomised 716 pre- and peri-menopausal women with lymph node-positive tumours to either six cycles of FEC-120 (epirubicin 60 mg/m^2, repeated days 1 and 8; oral cyclophosphamide 75 mg/m^2 /d, days 1-14; 5-fluorouracil 500 mg/m^2, i.v. days 1 and 8; q 28 days) or classical CMF [14]. The median age of the study population was 45 years. Approximately 60% of patients had 1-3 involved nodes, and approximately 40% had ≥4 nodes involved. Patients in the epirubicin-treated group had a significantly increased 5-year disease-free survival rate (62% versus 53%) and an increased 5-year overall survival (77% versus 70%) compared with those treated with classical CMF (Figure 1a). The HR for disease-free survival was 0.76, p=0.021; HR for overall survival was 0.71, p=0.043.

FRE-GFEA-05

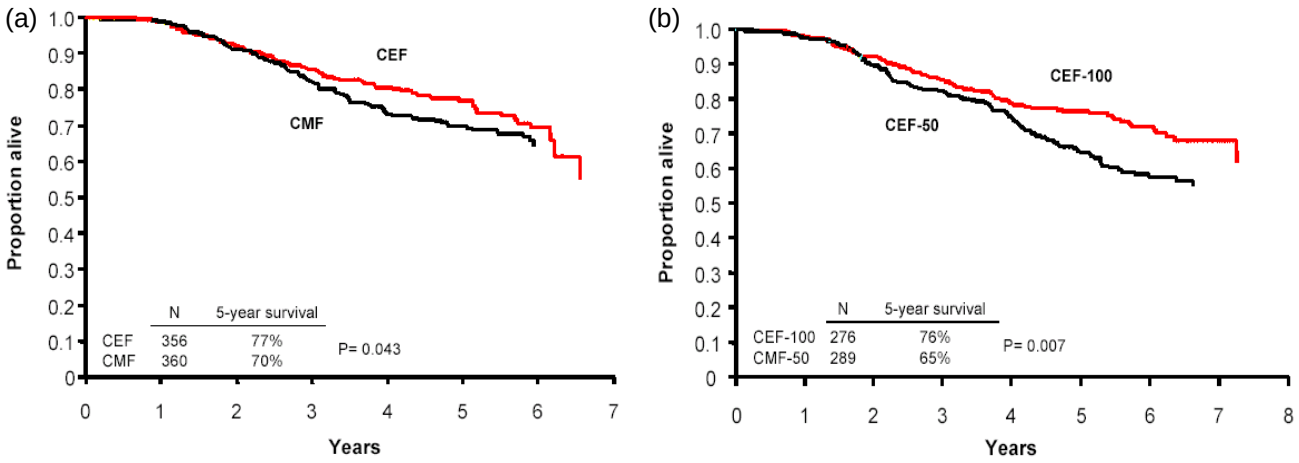
This second study (compared a higher dose epirubicin-containing regimen (FEC-100; epirubicin 100 mg/m^2 i.v., cyclophosphamide 500 mg/m^2 i.v., 5-fluorouracil 500 mg/m^2 i.v., q 21 days) with a lower dose epirubicin-containing regimen (FEC-50; epirubicin 50 mg/m^2 i.v.; cyclophosphamide 500 mg/m^2 i.v.; 5-fluorouracil 500 mg/m^2 i.v.; q 21 days) [15]. The study involved 565 pre- and postmenopausal women with either ≥4 nodes involved, or 1-3 positive nodes if tumours were ER-negative/progesterone receptor (PgR)-negative, with a histological grade of 2 or 3. Approximately 17% of the study population had 1-3 positive nodes and 80% of patients had ≥4 involved lymph nodes. The median age was 51 years and approximately half of the patients were post-menopausal. Patients treated with the higher-dose epirubicin regimen had a significantly greater 5-year disease-free survival rate (65% versus 52%, log-rank p=0.007) and 5-year overall survival (76% versus 65%, log-rank p=0.007; Figure 1b) than patients given the lower-dose epirubicin regimen. The overall reduction in risk of relapse was 32% (HR = 0.68). The relative reduction in the risk of death was 31% (HR = 0.69).

Although neither trial was powered for subset analyses, improvements in disease-free survival and overall survival were observed both in patients with 1-3 positive nodes and in those with ≥4 nodes involved when comparing the FEC-120 or FEC-100 groups with their respective controls. Furthermore, in the dose intensity study, similar improvements in disease-free survival and overall survival were observed in both pre- and postmenopausal women treated with FEC-100 compared with FEC-50.

Implications of MA-5 and GFEA-05 for optimal epirubicin dosing

The available data therefore support the existence of a clinically relevant dose-response curve for epirubicin in adjuvant therapy, and are consistent with the results of other studies, which have shown that FEC-50 is no better than, and may be inferior to, classical CMF, for example [16]. However, the use of FEC-120 was associated with a difficult level of side effects [14] in particular, an 11% incidence of neutropenic fever, despite prophylactic antibiotics, compared with 2% for classical CMF. Therefore, with cyclophosphamide 600 mg/m^2 an epirubicin dose of 90 mg/m^2 looks safer [17] and seems close enough to optimal dose intensity for general use in future studies.

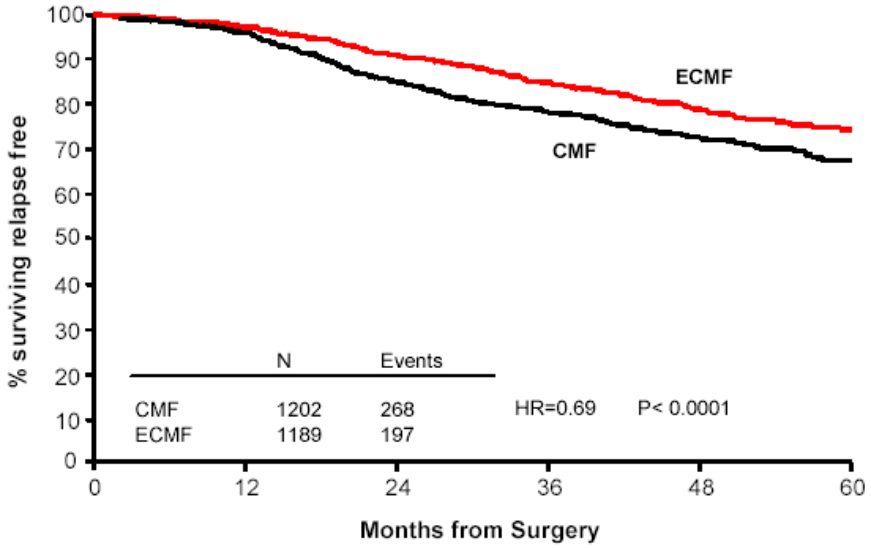
Figure 1: **5-year overall survival curves (all patients) for (a) MA-5 and (b) GFEA-05.**



UK National Epirubicin Adjuvant Trial Meta-Analysis (*NEAT* and Mc*NEAT* (BR9601))

A pre-planned interim meta-analysis of *NEAT* and BR9601 was presented at ASCO June 2003 [18], and largely confirm the efficacy of adequately dosed epirubicin as shown in NCIC MA-5, albeit with less toxicity as part of a sequential regimen, employing 4 cycles of epirubicin 100 mg/m^2 followed by 4 cycles classical CMF (Figure 2). These data would appear to vindicate the epirubicin doses adopted in ***tAnGo***.

Figure 2: **Disease-free survival curves for NEAT/McNEAT (all patients; 456 events)**

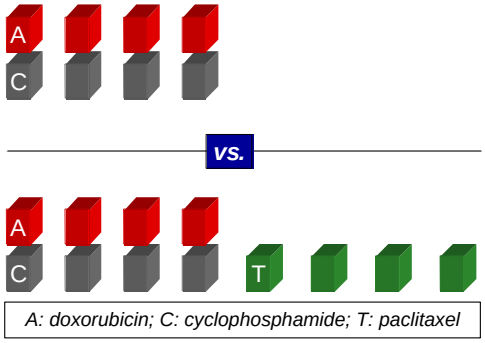


5.5 Taxanes in adjuvant therapy

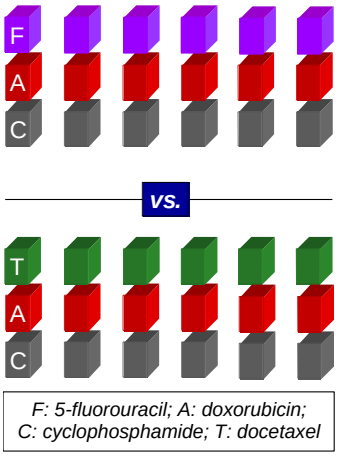
At the time of writing, data have been reported from three large phase III trials addressing the use of taxanes in adjuvant therapy for breast cancer, namely: CALGB 9344, NSABP B28, and BCIRG 001. Both CALGB 9344 and NSABP B28 are 3000-patient trials which follow a similar design and address the sequential addition of single agent paclitaxel to a standard regimen of doxorubicin and cyclophosphamide (AC). The BCIRG 001 trial on the other hand evaluates docetaxel in combination with doxorubicin, and cyclophosphamide (TAC) using the established combination of 5-fluorouracil, doxorubicin, and cyclophosphamide (FAC) as a direct comparator. Figure 3 shows the randomisation schema for these trials.

Figure 3: **A simplification of (a) the CALGB 9344 and NSABP B28 trial designs and (b) the BCIRG 001 trial design.**

(a)



(b)



CALGB 9344

To date, 4 separate analyses of the CALGB 9344 data have been presented and these are summarised in Table 2.

***Table 2*: CALGB 9344: Summary of intent-to-treat analyses reported to date.**

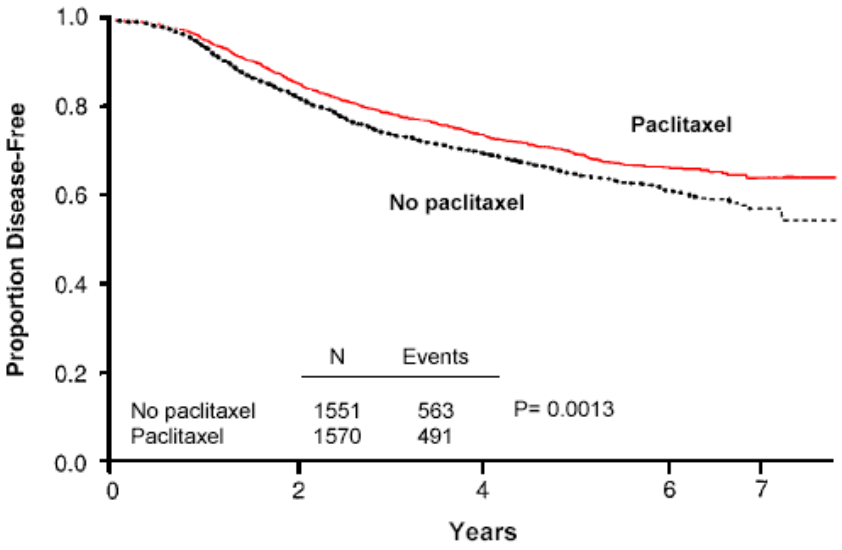
Median follow-up (months)	**21** [19]	**30** [20]	**52.5** [21]	**69** [2]
No. of events	453	624	901	1054
Risk of relapse (HR)	0.79	0.78	0.87	0.83
95% C.I.	0.66-0.95	0.67-0.97	0.76-0.99	0.73-0.94
p	0.0013	0.0026	0.031	0.0013
No. of deaths	200	342	589	742
Risk of death (HR)	0.77	0.75	0.86	0.82
95% C.I.	0.58-1.02	0.60-0.93	0.73-1.02	0.71-0.95
p	0.050	0.0076	0.062	0.0061

The first pre-planned analysis of this trial was presented at ASCO in 1998 with 453 events and 200 deaths [19]. At this time, the median follow up was just 21 months. The event rate reflected the high-risk population recruited and the large size of the trial. The addition of four cycles of paclitaxel was shown to reduce the risk of recurrence by 21%, compared with control (HR = 0.79, 95% C.I. 0.66-0.95, p=0.0013). Paclitaxel also provided a 23% reduction in the risk of death (HR = 0.77, 95% C.I. 0.58-1.02, p=0.05).

An additional unplanned interim analysis was carried out at the request of the FDA at 30-months median follow-up (624 events; 342 deaths) and this was reported in September 1999 [20]. The risk reduction reported in this analysis remained the same as in the 453-event analysis and formed the basis of a successful FDA Oncology Drugs Advisory Committee application. Given the size of the paclitaxel-related benefit in both disease-free and overall survival, it seemed highly implausible, with reference to the 1998 Oxford Overview analysis, that this advantage might have been due to an increased treatment duration effect alone. Paclitaxel is now licensed for adjuvant therapy of node-positive, early stage breast cancer in the USA.

The second planned CALGB 9344 analysis was carried out at 901 events (median follow-up of 52.5 months) and was presented at the NIH Consensus Conference on Early Breast Cancer in November 2000 [21]. Prior to publication of these data, a further updated analysis was undertaken with 69 months median follow-up (1054 events; 742 deaths) and was presented in the Presidential Symposium at ASCO May 2002. This data set has now been published [2] confirming the survival advantage reported in earlier analyses, with a 17% reduction in the risk of relapse (HR = 0.83, 95% C.I. 0.73-0.94, p=0.0013; Figure 4) and an 18% reduction in the risk of death (HR = 0.82, 95% C.I. 0.71-0.95, p=0.0061) on intent-to-treat analyses.

***Figure 4*: Disease-free survival curves for CALGB 9344 (all patients; 1054 events)**
(Reprinted from ref. 2 with permission from the American Society of Clinical Oncology.)



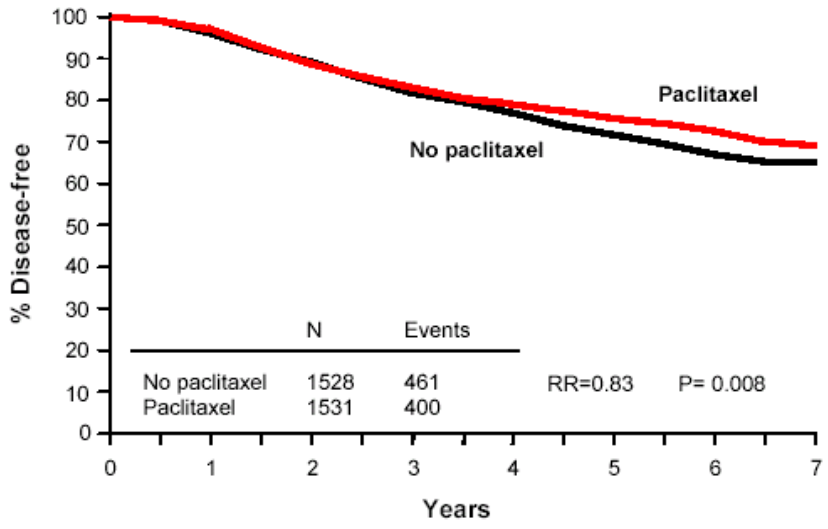
NSABP-B28

This trial has a similar design and size to CALGB 9344. In respect of chemotherapy, B28 differs only in employing a paclitaxel dose of 225 mg/m^2 (cf. 175 mg/m^2). Preliminary data were reported at the NIH Consensus Meeting (Washington, November 2000) [21] with a median follow-up of 34 months. This was an unplanned analysis, undertaken at the invitation of the Consensus Conference. In contrast to CALGB 9344, they revealed no statistically significant advantage in either disease-free (HR = 0.93; 95% C.I 0.76-1.10) or overall survival (HR = 1.00; 95% C.I. 0.78-1.27) attributable to the addition of paclitaxel. However, in comparing these trials, important differences in patient characteristics and treatment administration emerge. NSABP B28 enrolled a lower risk population with just 30% of patients with ≥4 lymph nodes involved, as opposed to 54% in CALGB 9344. Furthermore, 66% of patients in B28 had ER-positive tumours, compared with 59% in 9344. As regards treatment differences, only 75% of patients in NSABP B28 received chemotherapy doses complying with protocol (cf. 85% in CALGB 9344). More patients also received tamoxifen in NSABP B28, a total of 84%, reflecting NSABP guidelines for offering tamoxifen to all post-menopausal women and this compares with a figure of just 60% in 9344. Furthermore, chemotherapy and tamoxifen were given concurrently in NSABP B28 compared with sequentially in CALGB 9344. Taken together, these factors may account for the ostensible differences in the results of these two studies, in respect of their early analyses, and the lower event rate in NSABP B28 (540 relapses and 253 deaths at a median follow-up of 34 months, compared with 624 relapses and 342 deaths at just 30 months in CALGB 9344).

An updated analysis of NSABP B28 was presented at ASCO June 2003 [3]; this was the first *pre-planned* analysis. Of 1528 patients randomised to AC, just 461 had relapsed or died, with 255 deaths; of the 1531 patients randomised to AC→T, 400 had relapsed or died, with 243 deaths. The HR for disease-free survival was 0.83 (95% C.I.: 0.73-0.95; p=0.008; Figure 5); the HR for overall survival was 0.94 (95% C.I.: 0.78-1.12; p=0.46).

At 64.5 months median follow-up, 76% of patients randomised to AC→T are alive and disease-free, and 72% of those allocated AC. There is no trend towards selective paclitaxel relapse-free or disease-free survival advantage in patients with ER-negative tumours, and no significant treatment interaction with age, tamoxifen treatment, nodal status, or tumour grade.

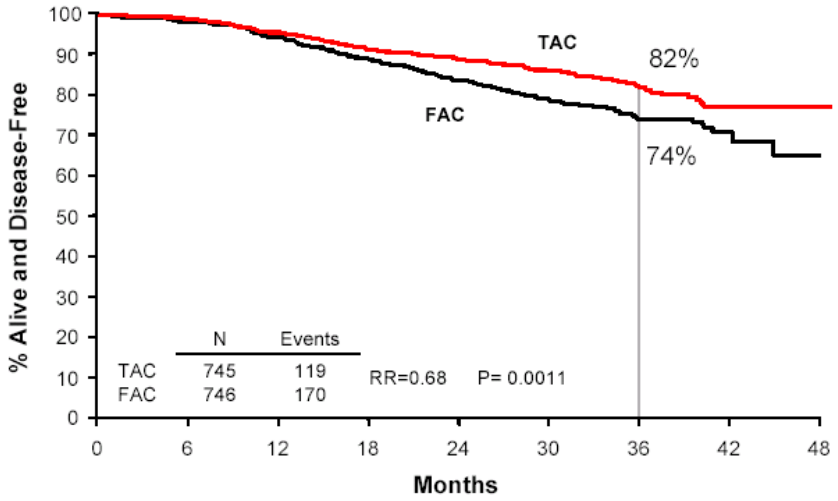
Figure 5: **Disease-free survival curves for NSABP B28 (all patients; 861 events)**



BCIRG 001

The use of a taxane (in this case docetaxel) in simultaneous combination is addressed by this trial which compared six cycles of FAC (5-fluorouracil 500 mg/m^2, doxorubicin 50 mg/m^2, and cyclophosphamide 500 mg/m^2) with six cycles of TAC (docetaxel 75 mg/m^2, doxorubicin 50 mg/m^2, and cyclophosphamide 500 mg/m^2). The first pre-planned interim analysis of this study was presented at ASCO, May 2002 with 33 months median follow-up (289 events; 133 deaths) [22]. These data suggest that TAC is associated with a statistically significant reduction in the risk of recurrence of 32% (HR = 0.68, 95% C.I. 0.54-0.86, p=0.0011; Figure 6). Comparison of overall survival data between treatment groups currently demonstrates no statistically significant difference. However, the substitution of docetaxel for 5-fluorouracil is associated with a 24% reduction in the risk of death (HR = 0.76, 95% C.I. 0.54-1.07, p=0.11).

***Figure 6*: Disease-free survival curves for BCIRG 001 (all patients; 289 events)**



Taxanes in neo-adjuvant therapy for breast cancer

To date, three phase III randomised trials have reported data on the incorporation of taxanes in neoadjuvant therapy and these entirely support the adjuvant canon. Each of these trials addresses the sequential use of single agent taxanes as consolidation (NSABP B27; Aberdeen trial) or induction (M.D. Anderson) for anthracycline-based combination regimens. Response and disease-free survival data for the three trials are shown in Table 3. Their designs are also detailed below.

***Table 3*: Summary of neoadjuvant taxane trials**

Study	Regimen	OR (%)	PR (%)	DFS (%)
NSABP 27 [23], [24]	AC × 4 + surgery AC × 4 + surgery + docetaxel AC × 4 + 4 × docetaxel + surgery	85 91	13.7 25.6	Not reported Not reported
Aberdeen trial [25]	CVAP × 4 + docetaxel × 4 CVAP × 8	94 66	34 13	90 at 3 yrs 77 at 3 yrs
M.D. Anderson trial [26]	paclitaxel × 4 + surgery + FAC × 4 FAC × 4 + surgery + FAC × 4	80 79	14 23	86 at 4 yrs 83 at 4 yrs

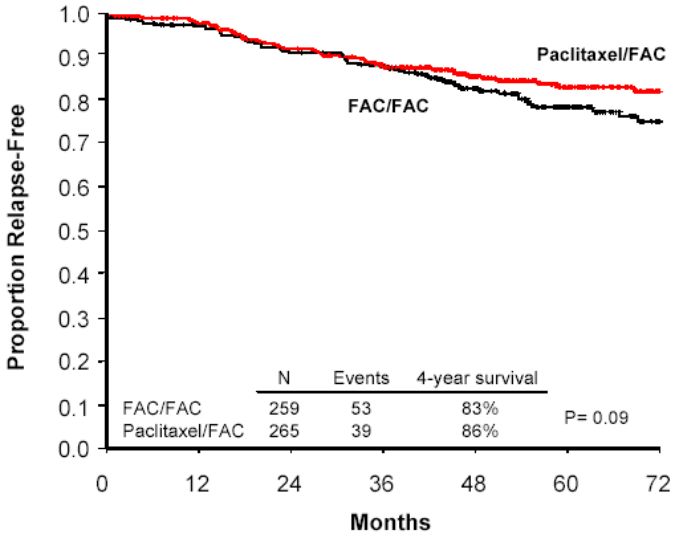
OR: Overall response; PR: Partial response

NSABP B27: This 2500 patient study addressed the sequential addition of four cycles of single agent docetaxel (100 mg/m^2) in both the neoadjuvant and adjuvant settings, after four cycles of AC (doxorubicin 60 mg/m^2; cyclophosphamide 600 mg/m^2). As yet, only response data have been reported for the neoadjuvant arms of the trial.

Aberdeen trial: Like NSABP B27, this trial evaluates the sequential addition of four cycles of single agent docetaxel 100 mg/m^2 to four cycles of an anthracycline-based regimen, in this case CVAP (cyclophosphamide, vincristine, doxorubicin, prednisolone), by a direct comparison with 8 cycles of neoadjuvant CVAP. A total of 145 patients were evaluated for clinical response.

M.D. Anderson trial: Four cycles neoadjuvant single agent paclitaxel were compared directly to 4 cycles neoadjuvant FAC. Both arms subsequently received four cycles adjuvant FAC. The response data are shown in Table 3. These data have been presented together with disease-free survival from a parallel adjuvant study. The combined data set comprised a total of 524 patients, of who 174 received the neoadjuvant regimen, and 350, the adjuvant. At a median follow-up of 43.5 months (range: 5-71 months), there is a clear trend towards fewer events in those patients treated with paclitaxel (Figure 7). This advantage emerges earlier in the ER-poor sub-group than in those patients with ER-positive tumours (see Figures 9a and 9b).

***Figure 7*: Disease-free survival curves for the M.D. Anderson trial (all patients; 92 events)**

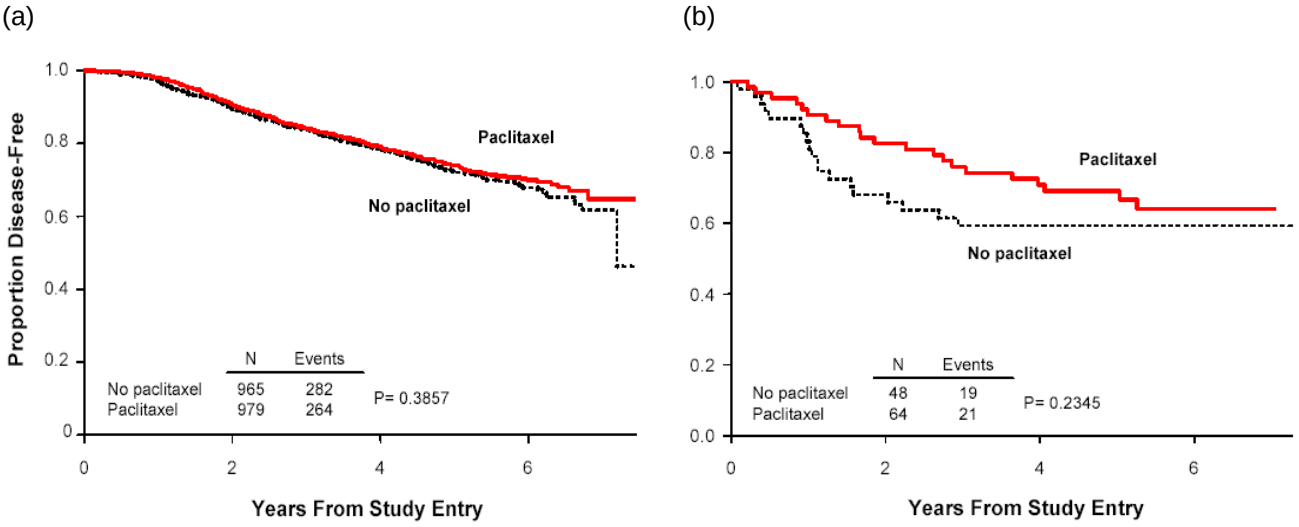


Taxanes and hormone receptor status

The 30-month median follow-up analysis of CALGB 9344 included an unplanned retrospective sub-group analysis based on hormone receptor status [27]. The results of this sub-group analysis provoked considerable debate as they suggested that most of the treatment benefit (attributable to paclitaxel within the trial population as a whole) might be limited to the 1055 patients with hormone receptor negative (or unknown) status. In this subgroup, paclitaxel conferred a reduction in risk of 32% (HR = 0.68; 95% C.I. 0.55-0.85) compared with only 8% (HR = 0.92; 95% C.I. 0.73-1.16) for patients with hormone receptor positive tumours. Similar data for recurrence are reported in the most recent CALGB 9344 disease-free survival data reports [2]; receptor negative/unknown: HR = 0.72, 95% C.I. 0.59-0.86; receptor positive: HR = 0.91, 95% C.I. 0.78-1.07. A similar trend was seen in early NSABP B28 with a HR of 0.75 seen for paclitaxel-treated patients with ER-poor tumours [3].

With greater maturity, and further events, it seems plausible that the advantage for paclitaxel apparent in patients with receptor-negative tumours in CALGB 9344 might extend to receptor-positive patients too. Exploratory subset analyses of patients with ER-positive tumours who, inadvertently, were not treated with tamoxifen, are also intriguing, and indicate a trend towards taxane benefit in these circumstances (Figure 8).

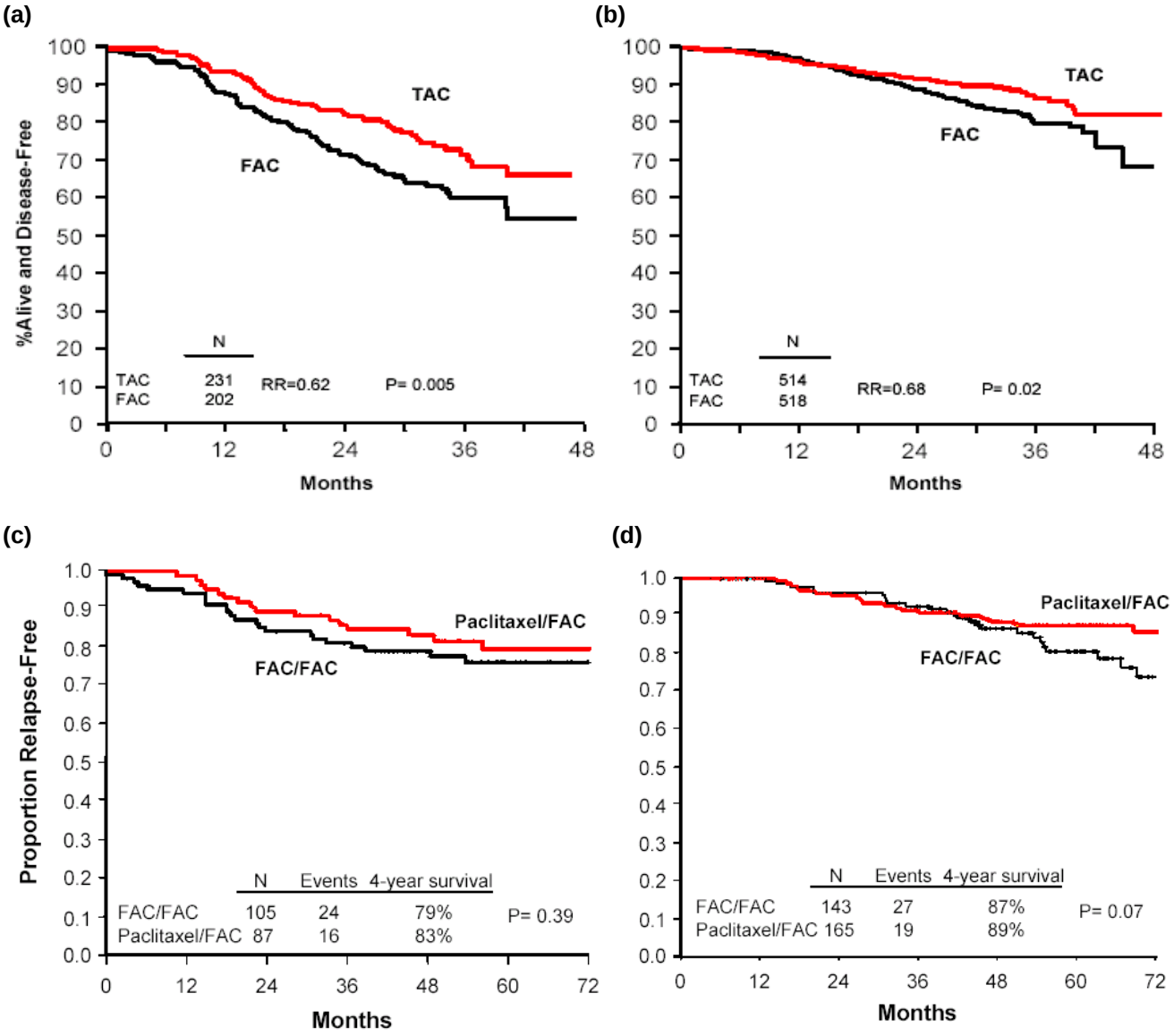
***Figure 8*: CALGB 9344: Disease-free survival curves for patients with (a) ER-positive tumours who received tamoxifen and (b) did not receive tamoxifen.**



This raises the possibility that at least in the early phase of follow-up, taxanes may have similar effects to tamoxifen in complementing the benefits of conventional chemotherapy and providing quantitatively similar degrees of non-cross resistance, particularly in those patients with p53 mutant tumours perhaps. The more extensive use of tamoxifen in B28 may also have implications here; certainly, a proportion of the "hormone receptor-negative" patients in 9344 (34% of the total trial population) might have been expected to gain some modest benefit from tamoxifen. Some of these would almost certainly be classed as ER-weak using modern terminology, with Q scores of up to 4 - 5.

These data are consistent with the burden of evidence emerging from other studies of taxanes in early stage breast cancer. In the M.D. Anderson trial, for example, we see the taxane advantage emerges earlier in patients with ER-poor tumours, the same trend being apparent only later in those with ER-positive tumours [26] (Figures 9a and 9b). The BCIRG 001 data analysed by ER status are also supportive, and unlike both CALGB 9344 and NSABP B28, these subgroup analyses were prospectively defined (ER and PgR negative vs. ER and/or PgR positive) [22]. Again, the taxane advantage emerges later in ER/PgR-positive patients (Figure 9c and 9d).

***Figure 9*: Disease-free survival curves for ER-negative patients in BCIRG 001 (a) and M.D. Anderson trial (c), and for ER-positive patients in BCIRG 001 (b) and M.D. Anderson trial (d).**



Faced with this emerging body of evidence, the ***tAnGo*** trial Steering Committee (November, 2002) unanimously elected to modify the ***tAnGo*** entry criteria to include all patients with early stage breast cancer, irrespective of tumour ER or PgR status.

5.6 Rationale for the development of the AC-T regimen

Justification for the use of an anthracycline-based, paclitaxel-containing stem as the basis for developing more effective adjuvant regimens. Of the taxane-containing regimens yet reported, the CALGB 9344/NSABP B28 research arm intuitively offers the easiest facility for such development. Further improvement of this regimen might be achieved by the following:

- Substitution of epirubicin for doxorubicin to improve the therapeutic index, by reducing the risk of cardiotoxicity (see Appendix 2) [28,29]. Whilst there is no evidence of a dose-response curve for doxorubicin between 60 and 90 mg/m^2, there is a clear dose-response relationship for epirubicin in the range 50 mg/m^2 to 100 mg/m^2 [15].
- Incorporation of newer, non-cross-resistant cytotoxics, in combination with paclitaxel during the sequential consolidation phase of treatment [6]
- The evaluation of newer targeted agents such as trastuzumab, tyrosine kinase inhibitors, matrix metallo-proteinase inhibitors (MMPIs), or vaccines, in a simple bifactorial maintenance therapy design [30]

5.7 Rationale for selecting gemcitabine for combination with paclitaxel

Newer drugs with promising single agent activity in advanced breast cancer include capecitabine, vinorelbine, gemcitabine and trastuzumab. However, although they are widely used as single agents, feasibility studies indicate that the combination of either vinorelbine [31-37] or capecitabine [38] with paclitaxel is unacceptably toxic for the adjuvant context, and that gemcitabine may have a superior therapeutic index in this respect.

Gemcitabine: single agent activity in breast cancer

Eight phase II trials of gemcitabine in patients with advanced breast cancer have shown single agent activity, with objective responses in the range 14-42%. These studies are summarised in Table 4.

***Table 4*: Gemcitabine activity in metastatic breast cancer: single agent phase II trials**

Author	Year	*n*	% Patients having previous chemotherapy		C.R. %	P.R. %	O.R. %
			adjuvant	met.[1st, 2nd line etc.]			
Carmichael [39]	1995	40	18	48[a]	7.5	17.5	25
Blackstein [40]	1997	35	60		11	26	37
Spielmann [41]	1997	43	33[f]	68[b]	9	18	28
Akrivakis [42]	1999	13			7.5	7.5	15
Possinger [43]	1999	42	24		0	14	14
Schmid [44]	1999	20		25[a], 55[b]	5	21	25
Brodowicz [45]	2001	25		36[a,f],64[f,b]	4	12	16
Gerson [46]	2001	19	10.5	32[a],32[c],16[c],5[d],5[e]	10.5	31.5	42

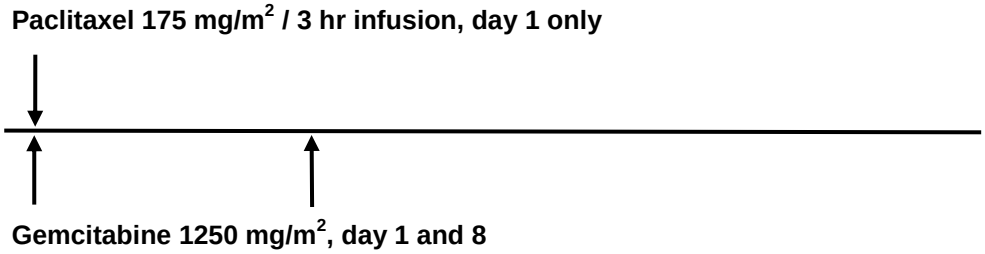
a: 1st line; *b*: 2nd line or higher; *c*: 3rd line; *d*: 4th line; *e*: 5th line; *f*: previous anthracycline
CR: Complete response; PR: Partial response; OR: Overall response;

Gemcitabine and paclitaxel: phase I trials; schedule feasibility

Besides their demonstrable single agent activities, gemcitabine and paclitaxel have different mechanisms of action and this may provide potentially useful non-cross resistance. Furthermore, they have essentially non-overlapping toxicities. There are also suggestions of a synergistic interaction as paclitaxel may increase intracellular accumulation of dFdCTP, the active metabolite of gemcitabine, and this may in turn enhance anti-tumour activity [47].

The combination of gemcitabine and paclitaxel has been thoroughly evaluated in a number of different schedules by several groups in phase I and phase II studies. Phase I studies in patients with recurrent ovarian cancer previously treated with platinum-based chemotherapy, have defined a 3-week regimen offering paclitaxel day 1 and gemcitabine days 1 and 8 (n=12 patients) as entirely feasible and generally well tolerated (Figure 10). These were carried out at the Universities of Birmingham, Leeds, and Lund, 1996 to 1998 [48,49].

Figure 10: **Gemcitabine and paclitaxel dose/ schedule recommended for further study**



These investigations also showed a number of other schedules to be unfeasible. These included a 28-day cycle with gemcitabine days 1, 8, and 15, with paclitaxel day 8, (n=9), and a 21-day cycle with gemcitabine days 1 and 8 and paclitaxel day 8 (n=27). Both these treatment schedules were confounded by myelotoxicity requiring dose omission and/or treatment delays. However, taking the study as a whole, and all three treatment schedules, 77% of patients (n=30) completed six cycles of chemotherapy; just 13% (n=5) discontinued because of disease progression; 8% (n=3) stopped due to adverse events; and 3% (n=1) for personal reasons. The maximum tolerated dose for the paclitaxel day 1 and gemcitabine days 1 and 8 regimen was not established; however, the dose recommended for further study was defined, with both drugs given at 'full' dose, paclitaxel 175 mg/m^2/3 hr and gemcitabine 1250 mg/m^2/30 min.

The laboratory toxicities encountered using this schedule are shown in Table 5. Non-haematological toxicity included chills (four patients), phlebitis (four patients), chest pain (two patients), and dry skin (two patients). Other less common adverse events comprised asthenia, injection site pain, flu syndrome, and myalgia. Although pharmacokinetic studies showed no interference, transaminase rise seemed more common when gemcitabine was given first. Paclitaxel was therefore administered before gemcitabine on day 1 throughout the subsequent part of the study, and this sequence is recommended for the ***tAnGo*** trial. No neutropenic sepsis was encountered.

Table 5: **Laboratory toxicities for paclitaxel day 1 and gemcitabine days 1 and 8 (as assessed days 1, 8, or 15)**

Parameter	**Overall (n = 12)**		**Dose Level 1: gemcitabine 1250 mg/m^2 & paclitaxel 150 mg/m^2 (n = 3)**		**Dose Level 2: gemcitabine 1250 mg/m^2 & paclitaxel 175 mg/m^2 (n = 9)**	
	Grade 3 n (%)	Grade 4 n (%)	Grade 3 n (%)	Grade 4 n (%)	Grade 3 n (%)	Grade 4 n (%)
Neutropenia	6 (50.0)	5 (41.7)	1 (33.3)	1 (33.3)	5 (55.6)	4 (44.4)
Leucopenia	7 (58.3)	1 (8.3)	2 (66.7)	0	5 (55.6)	1 (11.1)
Thrombocytopenia	1 (8.3)	1 (8.3)	1 (33.3)	0	0	1 (11.1)
Hyperglycemia	7 (58.3)	0	2 (66.7)	0	5 (55.6)	0
ALT	4 (33.3)	0	0	0	4 (44.4)	0

Other phase I trials adopting paclitaxel day 1, gemcitabine days 1 and 8

At least one other phase I trial has been published in abstract form addressing this combination, plus a further dose-ranging phase II study. In a phase I study reported by Rinaldi *et al*. [50], 29 patients with advanced solid tumours were treated with 89+ cycles at five dose levels of gemcitabine and paclitaxel (see Table 6). In each case, the dose limiting toxicity encountered was febrile neutropenia. Non-haematological toxicity was mild with no grade 3 or 4 problems encountered. There were no toxic deaths. Maximum tolerated dose was not defined. The authors proposed closing the study after a total of 10 patients were treated at dose level 5.

Table 6: **Summary of toxicity data reported by Rinaldi *et al*. [50].**

Dose level	Gemcitabine mg/m^2	Paclitaxel mg/m^2	*n*	DLT
1	800	150	6	1
2	1000	150	6	1
3	1000	175	4	0
4	1000	200	3	0
5	1300	200	8+	1

Phase II/dose-ranging studies adopting paclitaxel/ 3hr infusion day 1, gemcitabine days 1 and 8.

This schedule has been evaluated by several other groups in a number of different tumour types. Their findings are summarised below in Table 7. These include five phase II studies and a further dose-ranging study, which adopted fixed dose gemcitabine. In total, these other studies report on 366 patients, with a risk of neutropenic sepsis varying from 0 – 7%.

Table 7: **Other trials utilising gemcitabine 1000-1100 mg/m^2 days 1 and 8, with paclitaxel 175-200 mg/m^2 day 1, q 21/7**

Tumour	Gemcitabine mg/m^2	Paclitaxel mg/m^2	*n*	No. of cycles	Grade 4 ANC (%)	Neut. sepsis (%)	Grade 4 Platelet (%)	Non-haematol. toxicity (%)
NSCLC [a]	1000	200	54	274	5	2	0.7	6 *(G3; neuro)*
NSCLC [b]	1000	200	164	na	10.5	na	1.2	6.2 *(G3; neuro)*
NSCLC [c]	1000	175	20	na	na	none	na	5 *(G4; neuro)*
NSCLC [d]	1000	150/175/ 200 esc$^{n.}$ 200 fixed	30 30	214	12	3.5	3.5	1.7 *(TTP)* 1.7 *(G3; pulm.)*
Breast [e]	1000	175	24	137	7	7	5.4 (*G3+4)*	7 *(G3; neuro)*
Head & neck [f]	1100	200	44	205	21 *(G3/4)*	5 *(G3/4)*	3.5	2 *(G3/4)*

Sources: ***a****:* Monnier *et al*., 2000 [51]; ***b****:* Kosmidis *et al*., 2000 [52]; ***c****:* Auerbach *et al*., 2000 [53]; ***d****:* Giaccone *et al*., 2000 [54]; ***e****:* Murad *et al*. 2000 [55]; ***f****:* Fountzillas *et al*., 2000 [56].

Gemcitabine and Taxanes: Phase II trials

A further three phase II studies have addressed the activity of gemcitabine in combination with paclitaxel and a further six in combination with docetaxel. For paclitaxel, these studies reported impressive overall response rates of between 45 and 68% and CR rates in the of 16 to 21%, in a variety of schedules, clinical contexts and previous treatment entry criteria. These figures are encouraging as such high CR rates are arguably unusual, especially in heavily pre-treated patients, most of whom had received prior anthracyclines and some, even taxanes. These data are summarised in Table 8.

Table 8: **Gemcitabine activity in metastatic breast cancer: phase II trials in combination with taxanes.**

Author	Year	*n*	% Patients having previous chemotherapy		C.R. %	P.R. %	O.R. %
			adjuvant	met. 1st, 2nd line etc.			
Gemcitabine & paclitaxel							
Sanchez [57]	1998	44	93[c]	20[d]	16	30	45
Colomer [58]	2001	43	72		21	47	68
Murad [54]	2001	29	n/a	n/a	17	38	55
Gemcitabine & docetaxel							
Mavroudis [59]	1999	52	44	(52[a,c], 48[b,c]) *d for 48%*	14	40	54
Fountazilas [60]	2000	39	8	51	8	28	36
Brandi [61]	2001	30	17[f]	n/a	7	53	60
Laufman [62]	2001	39	82	23	5	75	79
Kornek [63]	2002	51		27[a,(c for 20%)]	10.5	50	60.5
Pelegri [64]	2002	32	44[(c for 28%)]		12.5	53	35.5
Gemcitabine, epirubicin & paclitaxel							
Gennari [65]	2001	36			31	61	92

a: 1st line; *b*: 2nd line or higher; *c*: previous anthracycline; *d:* previous paclitaxel.

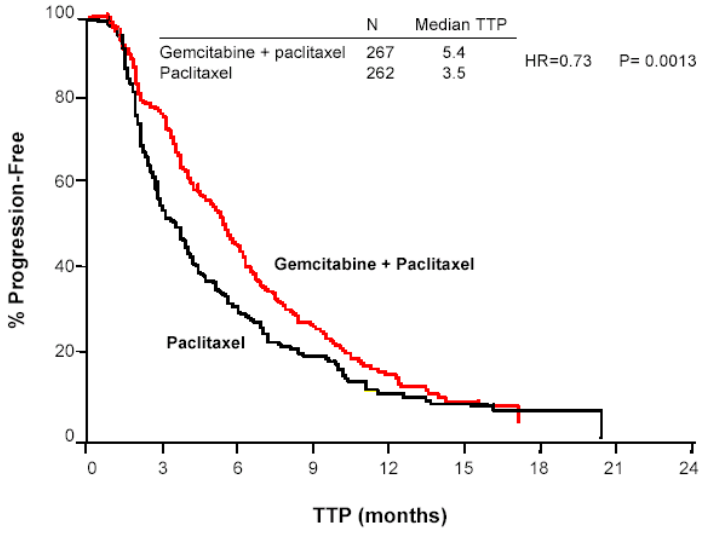
Gemcitabine and paclitaxel: pivotal phase III study

In a phase III randomised trial, 529 patients with metastatic breast cancer, previously exposed to anthracycline-based adjuvant therapy (but no prior chemotherapy for metastatic disease) were randomised to paclitaxel + gemcitabine versus paclitaxel alone, and treated until disease progression. In excess of 70% of patients had visceral metastases and 75% more than two sites of recurrence.

Data from a pre-planned interim analysis were presented at ASCO June 2003 [4], for which time to progression (TTP) was the primary endpoint. Median TTP for gemcitabine + paclitaxel was 5.4 months (95% C.I.: 4.6 – 6.1) and 3.5 moths for paclitaxel alone (95% C.I.: 2.9 – 4.0), p=0.0013 (Figure 11). The HR was 0.734 (95% C.I.: 0.607 – 0.889; p=0.0015), with a 50% improvement in probability of being progression-free at 6 months. There was a trend towards better pain control and global quality of life with gemcitabine + paclitaxel. CTC grade 4 haematological toxicity was more common with gemcitabine + paclitaxel than paclitaxel alone, (17.2% vs. 6.6%). There was one toxic death in each arm.

These data sustain the case for evaluating a gemcitabine-paclitaxel couplet, in adjuvant therapy for women with early stage breast cancer.

Figure 11**: Gemcitabine + paclitaxel in metastatic breast cancer: time to progression curves**



6. *tAnGo*: trial design

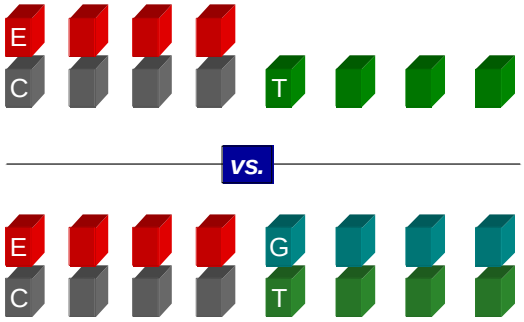
We propose a randomised, phase III clinical trial addressing the addition of gemcitabine (Gemzar®) to paclitaxel (Taxol®) in the consolidation phase of a modified epirubicin-substituted CALGB 9344 regimen. The design is shown below in Figure 12. We have chosen to substitute epirubicin (Pharmorubicin®; Ellence®) 90 mg/m^2 for doxorubicin 60 mg/m^2, in keeping with European practice, to minimise cardiotoxicity [14] .

Based on the preliminary retrospective sub-group analyses of CALGB 9344, which showed a benefit for paclitaxel only in ER-negative patients, ***tAnGo*** was originally designed as a study for patients with ER/PgR-poor tumours. The Steering Committee appreciated that this might well be an artefact of short follow-up, in that relapse events typically occur earlier in patients with ER-negative tumours. Mindful of this, the first edition of the protocol stated that the ER/PgR-poor entry qualification might be relaxed, were this justified by the emergence of data supporting a taxane advantage in patients with ER/PgR-rich tumours too.

This question was formally reviewed by the trial Steering Committee in November 2002. It was unanimously agreed that this change in entry criteria, to accept all patients with early stage breast cancer irrespective of tumour ER/PgR status, was now reasonable. The factors underpinning this decision were as follows:

1. The intent-to-treat analysis of CALGB 9344 was significantly positive for both disease-free survival (HR = 0.83, 95% C.I. 0.73-0.94, p=0.0023) and overall survival (HR = 0.82, 95% C.I. 0.71-0.95, p=0.0061) with a median follow-up of 69 months.
2. The ER-poor subgroup analyses of both CALGB 9344 and NSABP B28 were retrospective and not pre-planned. In contrast, analysis of the BCIRG 001 data by hormone receptor status was prospectively defined (ER and PgR negative vs. ER and/or PgR positive) and trends towards taxane benefit are now evident in the ER-positive subsets of BCIRG 001. They are also now evident in the M.D. Anderson trial. In each case, the taxane advantage emerges later in the ER-positive patients. More mature data from NSABP B28 presented at ASCO June 2003 vindicate this decision, showing a non-significant difference in paclitaxel effect in ER-poor (HR = 0.82) and ER-rich (HR = 0.90) subgroups for relapse-free survival.
3. The question being addressed in the ***tAnGo*** trial relates to the value of gemcitabine in adjuvant therapy. As a novel anti-metabolite, there is no *a priori* reason to suggest gemcitabine is likely to have any selective effect in ER-poor patients. Since ER status is a stratification variable for randomisation, we can prospectively address this question in the analysis of the ***tAnGo*** trial.

Figure 12: ***tAnGo*: trial design**



E, epirubicin (Pharmorubicin®; Ellence®)
C, cyclophosphamide
G, gemcitabine (Gemzar®)
T, paclitaxel (Taxol®)

Primary endpoint

- 5-year disease-free survival

Secondary endpoints

- 5- and 10-year overall survival
- 10-year disease-free survival
- Toxicity, dose-intensity and tolerability
- Serious adverse events

6.1 Eligibility criteria

Inclusion criteria

- Histological diagnosis of invasive breast carcinoma
- Completely resected early stage disease
- Definite indication for adjuvant chemotherapy
- Any nodal status
- Any hormone receptor status
- Fit to receive either of the trial chemotherapy regimens. Adequate bone marrow, hepatic, and renal function *****
- ECOG performance status of 0, 1, or 2 (see Appendix 7)
- Written informed consent
- No previous chemotherapy or radiotherapy
- Radiotherapy intent is known (this must be stated at the point of randomisation)
- Randomisation within 8 weeks of surgery, but ideally within 1 month
- No previous malignancy except basal cell carcinoma or cervical carcinoma *in situ*, unless disease-free for 10 years, after surgical treatment only
- Non-pregnant and non-lactating, with no intention of pregnancy during chemotherapy, and prepared to adopt adequate contraceptive measures if pre-menopausal and sexually active
- No concomitant medical or psychiatric problems that might prevent completion of treatment or follow-up

***** *Recommendations:*

- Hb > 9 g/dL; WBC > 3 $\times$ 10^9/L; platelets > 100 x 10^9/L
- Bilirubin within normal range
- AST/ALT $\leq$ 1.5 x normal
- Creatinine $\leq$ 1.5 x normal
- No active, uncontrolled infection

Exclusion criteria

- Any of the above criteria not satisfied

6.2 Staging investigations

Required staging investigations will be minimal in keeping with standard UK practice in breast cancer management. All patients should have a full blood count (FBC), biochemical screen, to include liver function tests and serum calcium, and a chest X-ray (CXR). Further staging investigations will be performed as clinically indicated, and an isotope bone scan and liver ultrasound are expected to be performed routinely in higher risk >3-node positive patients.

7. Treatment plan

7.1 Surgery

Patients may undergo breast conservation surgery with axillary clearance, or modified radical mastectomy, as per UK BASO guidelines (or equivalent national guidelines elsewhere). Ideally, patients should be randomised into ***tAnGo*** within 4 weeks of surgery, but will be accepted into the trial up to 8 weeks from date of operation.

7.2 Tamoxifen and endocrine therapy

It is recommended that any previous hormone replacement therapy (HRT) or tamoxifen therapy **is stopped** prior to commencing chemotherapy. For appropriate patients, tamoxifen will normally commence after chemotherapy completes and continue for 5 years. However, if uncertainty about the optimal duration of tamoxifen persists, then ***tAnGo*** patients would be eligible for studies such as aTTom or ATLAS. Conversely, if these duration studies establish the case for extended tamoxifen (beyond 5 years) within the lifetime of the ***tAnGo*** trial, then clinicians should heed these findings.

Note that the ***tAnGo*** protocol does not restrict the use of any form of hormonal manipulation in these patients. In particular, such patients will remain eligible for randomisation in other endocrine therapy trials, and adjuvant aromatase inhibitors are acceptable either within clinical trials or in the event of failure to tolerate tamoxifen. Details of ***tAnGo*** patients eligibility for randomisation into other studies are discussed later (see section 14). Note that aromatase inhibitors may be used in preference to tamoxifen off-study once agreed by national/international consensus statement.

Severe menopausal symptoms and HRT

In the event of difficult menopausal symptoms induced by chemotherapy etc., HRT may be used at clinician's discretion in line with local practice. ***tAnGo*** patients are also eligible for randomised trials addressing the quality of life benefits of HRT.

7.3 Chemotherapy

Patients will be randomised into one of two treatment arms:

Control arm

Epirubicin 90 mg/m^2, slow push into fast drip, day 1 only
Cyclophosphamide 600 mg/m^2, slow push, day 1 only
} × 4 cycles at 3-weekly intervals

Followed by:

Paclitaxel 175 mg/m^2, 3 hr infusion, day 1 only × 4 cycles at 3-weekly intervals

Versus

Research arm

Epirubicin 90 mg/m^2, slow push into fast drip, day 1 only
Cyclophosphamide 600 mg/m^2 , slow push, day 1 only
} × 4 cycles at 3-weekly intervals

Followed by:

Paclitaxel 175 mg/m^2 , 3 hr infusion, day 1 only
Gemcitabine 1250 mg/m^2, 0.5 hr infusion, days 1 and 8
} × 4 cycles at 3-weekly intervals

Note: *paclitaxel should be given prior to gemcitabine (see section 5.7 for rationale).*

Pre-chemotherapy investigations

The following investigations are expected to be undertaken routinely before each cycle of chemotherapy in both treatment arms: symptom review, toxicity review, physical examination, FBC, biochemical profile (including liver function tests and serum creatinine).

7.4 Other medication

Antiemetics

These may be given according to local practice. However, we do recommend a 5HT3 antagonist (e.g. granisetron 3 mg i.v., or ondansetron 8 mg i.v.) and dexamethasone 8 mg i.v., before epirubicin and cyclophosphamide chemotherapy, followed by domperidone 10-20 mg p.o. t.d.s. × 5 days, with dexamethasone 2 mg p.o. t.d.s. x 3 days only, afterwards.

H2-antagonists etc.

Ranitidine 150 mg p.o. b.d. × 7 days, or similar, may be necessary to prevent steroid induced dyspepsia.

Aperients

Aperients/glycerine suppositories will be occasionally required for relief or prophylaxis of granisetron-related constipation.

Prophylactic mouthwashes

We recommend Corsodyl or Difflam mouthwash p.o. b.d. throughout the period of anthracycline-containing chemotherapy, to limit the incidence of oral mucositis.

Prophylactic antibiotic therapy

Although routine antibiotic therapy has been required by some adjuvant protocols (e.g. Canadian NCI FEC 120, the TACT study, BCIRG 001) their utility remains unconfirmed. We consider the need for prophylactic antibiotics in the ***tAnGo*** study to be uncertain and their use is left to the discretion of the responsible clinician, for example after a previous episode of neutropenic sepsis. However, investigators are alerted to the zero level of on treatment mortality in the above mentioned adjuvant studies. Data from the 1500 patient *Significant* trial, addressing this question prospectively, are expected in mid-2004.

GCSF

We do not anticipate any routine requirement for the use of rhG-CSF. However, in patients who have suffered neutropenic sepsis and/or faced delays in re-treatment because of inadequate neutrophil counts, we would encourage their being approached with a view to randomisation into the SPROG study (contact the Scottish Cancer Therapy Network, details on back cover).

Paclitaxel pre-medication

5HT3 antagonists will not be needed in the majority of cases during the paclitaxel or gemcitabine and paclitaxel phase of treatment. Similarly, oral steroids may not often be required as anti-emetics at this time either, on account of the high dose intravenous steroids used as part of paclitaxel pre-medication, and the modest emetic toxicity of these cytotoxics. Note that high dose dexamethasone frequently causes temporary glucose intolerance. The following paclitaxel premedication regimen is recommended to minimise risks of acute hypersensitivity reactions:

Paclitaxel pre-medication:

- **Dexamethasone 20 mg i.v. slow push or short infusion in 100 mL normal saline 60 min before paclitaxel**
- **Ranitidine 50 mg i.v. or Nizatidine 100 mg i.v. 20 min before paclitaxel**
- **Chlorpheniramine 10 mg i.v. 10 min before paclitaxel**

Some clinicians may prefer to use oral dexamethasone 20 mg p.o. 6 and 12 hrs before treatment with paclitaxel. However, we are not aware of any data demonstrating improved efficacy over the i.v regimen above, the oral regimen is vulnerable to poor compliance, deviation or forgetfulness. Furthermore, the oral regimen entails a higher cumulative steroid dose, and an increased risk of complications.

7.5 Dose modifications in response to toxicity

The principal toxicities of the drugs used in standard and control arms are listed in the respective Appendices 1-4. Toxicity should be recorded according to the Common Toxicity Criteria (Appendix 8). We suggest the following dose modifications as guidelines. As regards to myelosuppressive indications, we refer below to both day 1 and nadir blood counts . However, nadir counts are not obligatory.

Epirubicin and cyclophosphamide: Patients suffering neutropenic fever should be dose-reduced on subsequent cycles to a value of 80% of standard (i.e. 20% dose reduction). If a nadir count is undertaken, and grade 4 neutropenia confirmed (in the absence of fever), prophylactic antibiotics may be used at the discretion of the responsible clinician. Dose reduction is not necessary in the absence of fever. Furthermore, nadir blood counts on asymptomatic patients are not mandatory.

Patients experiencing nadir platelet counts $\leq 20 \times 10^9$/L, neutropenic fever, or grades 3/4 non-haematological toxicity (e.g. mucositis) are candidates for dose-reduction to 80% of previous. Day 1 chemotherapy should be delayed until platelet counts are $\geq 100 \times 10^9$/L, ANC $\geq 1.0 \times 10^9$/L, and non-haematological toxicities have recovered to ≤ CTC grade 1. If delay is required, subsequent doses should be reduced to 80% of original thereafter. Note that where patients have required dose reduction in response to toxicity encountered with epirubicin and cyclophosphamide, it is anticipated that this will reflect some idiosyncratic sensitivity to these agents (e.g. reduced anthracycline clearance). We therefore recommend that such patients should not be routinely dose-reduced for their first cycle of paclitaxel ± gemcitabine unless there are particularly extenuating circumstances, and these should be discussed with the trials office.

Paclitaxel: It is not anticipated that paclitaxel 175 mg/m^2/3-hrs, 3-weekly will cause troublesome myelosuppression. However, the same rules apply as for EC (see above) in this event. Similarly, it seems unlikely that patients will develop significant neuropathic deficit, within four cycles of treatment, at the doses used here. However, occasional problems may be encountered especially in diabetics and other patients with microvascular disease or pre-existing occult neuropathic deficits. The paclitaxel dose should be reduced to 135 mg/m^2/24 hr in the event of neuropathy interfering with function (CTC grade 2) as assessed on day 22, and omitted in event of impairment of activities of daily living (CTC grade 3).

Paclitaxel and gemcitabine: Patients experiencing neutropenic fever should have subsequent paclitaxel and gemcitabine doses reduced to 80% of previous, and chemotherapy should be delayed until ANC $\geq 1.0 \times 10^9$/L. Thrombocytopenia, with nadir platelet counts $< 20 \times 10^9$/L, will require gemcitabine dose reduction only, to 80% of standard protocol doses and day 1 chemotherapy should be delayed until platelet counts are $\geq 100 \times 10^9$/L. The response to grade 3 non-haematological toxicity will depend on the side effect encountered: thus, for example, there will be no need to reduce concomitant gemcitabine doses for peripheral neuropathy; similarly, paclitaxel dose reduction would not be required in the event of pulmonary toxicity. However, all treatment will be delayed until non-haematological toxicities have recovered to ≤ CTC grade 1 and, if treatment delay is required, subsequent doses should be reduced to 80% of original.

Note that during the paclitaxel/gemcitabine phase of treatment, day 8 blood counts should not be used as determinants of treatment or not. Day 8 gemcitabine should usually be given irrespective of the day 8 neutrophil count, unless there is evidence of active infection.

Cardiac toxicity

This is not anticipated at the cumulative doses of epirubicin achieved in this protocol, namely 360 mg/m^2 (see Appendix 2). However, occasional patients with pre-existing cardiac pathology may develop problems, and investigators should be alert to this possibility. In the event of congestive cardiac failure developing, patients should be investigated and treated as appropriate. If confirmed, epirubicin should be discontinued, and other chemotherapy may be given at the discretion of the responsible clinician.

Hepatic toxicity: transaminitis

We suggest an 20% gemcitabine dose-reduction (i.e. to 80% of standard protocol dose), in the event of grade 3 transaminitis (AST/ALT 5-20 × ULN) on day of treatment, at clinician's discretion. There is no evidence to suggest that transaminitis affects gemcitabine clearance. We have been unable to substantiate early concerns about gemcitabine's potential for clinically significant hepatic impairment. However, please ensure that details of transaminitis are recorded (under any other toxicity) on the treatment forms.

Other toxicities

Any other grade 3 toxicity will require dose reduction to 80% of previous, with re-treatment delayed until residual problem is ≤ CTC grade 1.

Table 9: **Summary of dose modifications in response to toxicity**

Rx	Toxicity	Action	Other advice
EC	Neutropenic fever	Reduce next dose EC by 20%*, maintaining this dose reduction for subsequent EC cycles. Delay re-treatment until ANC $\geq 1.0 \times 10^9$/L	Use prophylactic antibiotics; if appropriate consider *SPROG*. Subsequent T±G cycles should commence at full protocol dose.
EC	Asymptomatic grade 4 neutropenia (nadir ANC $\leq 0.25 \times 10^9$/L)	No dose reduction. Delay re-treatment until ANC $\geq 1.0 \times 10^9$/L.	Prophylactic antibiotics may be used for remaining cycles at discretion. If appropriate consider *SPROG*.
EC	Persistent neutropenia, with day 22 ANC < 1.0×10^9/L	Delay re-treatment until ANC $\geq 1.0 \times 10^9$/L. Dose reduce subsequent EC cycles by 20%* if delay is greater than 7 days.	If appropriate consider *SPROG*. Subsequent T±G cycles should commence at full protocol dose.
EC	Nadir platelets $\leq 20 \times 10^9$/L	Reduce next dose EC by 20%*, maintaining this dose reduction for subsequent EC cycles. Delay re-treatment until platelets $\geq 100 \times 10^9$/L	Subsequent T±G cycles should commence at full protocol dose.
EC	Persistent thrombocytopenia, with day 22 platelets < 100×10^9/L.	Delay re-treatment until platelets $\geq 100 \times 10^9$/L. Dose reduce subsequent EC cycles by 20%* if delay is greater than 7 days.	If appropriate consider *SPROG*. Subsequent T±G cycles should commence at full protocol dose.
EC	Other grade 3/4 toxicities.	Reduce next dose EC by 20%. Delay until toxicity grade ≤ 1.	Subsequent T±G cycles should commence at full protocol dose.
T±G	Neutropenic fever	Reduce next dose T±G by 20%*, maintaining this dose reduction for subsequent cycles. Delay re-treatment until ANC $\geq 1.0 \times 10^9$/L	Use prophylactic antibiotics; if appropriate consider *SPROG*.
T±G	Asymptomatic grade 4 neutropenia (nadir ANC $\leq 0.25 \times 10^9$/L)	No dose reduction. Delay re-treatment until ANC $\geq 1.0 \times 10^9$/L.	Prophylactic antibiotics may be used for remaining cycles at discretion. If appropriate consider *SPROG*.
T±G	Persistent neutropenia, with day 22 ANC < 1.0×10^9/L	Delay re-treatment until ANC $\geq 1.0 \times 10^9$/L. Dose reduce subsequent cycles by 20%* if delay is greater than 7 days.	If appropriate consider *SPROG*.
T+G	Nadir platelets $\leq 20 \times 10^9$/L	Reduce next gemcitabine dose (not paclitaxel) by 20%, maintaining this dose reduction for subsequent cycles. Delay until platelets $\geq 100 \times 10^9$/L.	
T+G	Persistent thrombocytopenia, with day 22 platelets < 100×10^9/L.	Delay re-treatment until platelets $\geq 100 \times 10^9$/L. Reduce subsequent gemcitabine doses (not paclitaxel) by 20% if delay is greater than 7 days.	
T±G	Other grade 3/4 toxicities	Dose reduce subsequent cycles by 20%. Delay until toxicity grade ≤ 1.	
T±G	Neuropathy grade 2	Reduce remaining paclitaxel doses (not gemcitabine) to 135 mg/m^2/24 hrs.	
T±G	Neuropathy grade 3	Stop treatment with paclitaxel (not gemcitabine).	
T+G	Transaminitis grade 3 (ALT/AST $\geq$ 5-20 $\times$ ULN)	Reduce next gemcitabine dose (not paclitaxel) by 20% at clinician's discretion.	See note on previous page. We remain uncertain as to clinical necessity.

*** Depending on treatment allocation if patient is randomised into *SPROG.***

Paclitaxel-related, acute hypersensitivity reactions

Despite routine prophylaxis with antihistamines and steroids etc. 2-4% of patients will suffer acute hypersensitivity reactions to paclitaxel. These usually occur in the first 5-10 minutes of the first or second cycle. Adrenaline (1 mL/1:1000, i.m.) should be immediately available, as should antihistamines, dexamethasone, and oxygen. Whilst mild/moderate reactions may subside with further steroids and antihistamines, allowing successful re-challenge, this practice should be avoided if the severity of the initial reaction was such that adrenaline was required. Occasional deaths have been reported in this context.

Mild symptoms Skin rash, flushing, localised pruritus	Reduce infusion rate; treat with further i.v. antihistamines; monitor until recovery, then re-challenge
Moderate symptoms Generalised pruritus or rash, mild dyspnoea, mild hypotension	Stop paclitaxel infusion, treat with i.v. steroids and i.v. antihistamines; re-challenge after recovery
Severe symptoms Bronchospasm, generalised urticaria, angio-oedema, hypotension systolic < 80 mmHg	Stop paclitaxel infusion, treat with i.m. adrenaline 1 mL 1:1000, i.v. steroids and i.v. antihistamines

7.6 Radiotherapy

Radiotherapy will be given, if required, after chemotherapy in keeping with local practice, and/or the guidelines in Appendix 5. There is no evidence that radiotherapy given 3 to 4 weeks after the last gemcitabine exposure is likely to result in any more intense an acute or delayed reaction.

8. Pathology

Standard information will be collected on all patients from the local histopathology report. This will include data regarding pathological size, tumour grade, presence of vascular/lymphatic invasion within the tumour, the nearest resection margins, ER status, and the total number of axillary lymph nodes removed, and the number that contain metastatic deposits. In addition, we will record PgR status, HER2 status, necrosis within invasive tumour, and extracapsular axillary lymph node spread, where available. All centres are asked to nominate an interested pathologist willing to act as a local contact. Determination of HER2 status is recommended where resources allow; Investigators will be asked whether HER2 status is known at randomisation (stratification: +++; other; not measured).

8.1 Predictive marker studies

Detailed histopathological studies on tumour tissue will allow for potential analyses addressing the importance of conventional pathological factors (especially ER and PgR status), as well as a number of newer candidate predictive markers, using both conventional multivariate techniques, as well as neural network analysis. This pathology sub- study will be performed in collaboration with Professor Carlos Caldas' laboratory at Cambridge University (the Sponsors of this sub- study).

In summary, paraffin embedded tumour blocks will be collected retrospectively by the Birmingham trials office for all patients that have consented to participate in this sub-study. Requests for blocks will be sent to the named Pathologist at each centre. The Pathologist will be required to anonymise the blocks and dispatch these directly to Cambridge for analysis. Cambridge will be responsible for returning the blocks and paying for collection.

It is anticipated that the system will work as follows:

a. For each patient consented to the pathology sub- study, the Birmingham trials office will identify the hospital where the tumour block is held together with the histology report number from *tAnGo* pathology forms.

b. A collative list of the Pathology hospital(s) identified from the pathology forms for each randomising site will be sent to the data coordinator for that site requesting contact details of a named Pathologist at each Pathology hospital, whom Birmingham trials office may contact for tumour blocks.

c. Blocks will be requested in batches. The Birmingham trials office will generate two lists for requesting the tumour blocks:

- The first list will contain the patient trial number, initials, date of birth, histology report number, randomising hospital and responsible clinician's name. The Pathologists should use this list to retrieve the blocks from storage with reference to the histology report number and additional identifiers supplied for each patient. Pathologists must sign off all blocks retrieved from storage and then return the list immediately to Birmingham trials office. Where two or more histology report numbers are listed for a patient(s), it will be the responsibility of the Pathologist to establish which contains the primary tumour. No other pre- selection is required locally.
- The second list will contain only the patients' trial number and histology report number(s) in order to preserve patient confidentiality. Pathologists must sign- off all corresponding tumour blocks (as retrieved from the first list) and dispatch these to Professor Caldas' team in Cambridge in the padded envelopes provided. Prior to dispatch, Pathologists must remember to clearly indicate patient trial number on each respective block.

d. On arrival at Cambridge, the receipt of each block will be recorded using a tracking system shared by Birmingham trials office and Cambridge only. Blocks will be re-coded with a unique identifier (analysis number) in preparation for analysis. Cambridge will obtain 5 cores (1 core normal tissue and 4 cores cancer) for tissue microarray and 5µm sections for isolation and amplification of DNA and RNA analysis as well as HER-2 status determination.

e. Following removal of the cores, Cambridge will return the blocks (where requested) to the named Pathologist and arrange for financial recompense. Any queries with regard to payment or return of blocks should be addressed to Translational Research Co-ordinator on telephone 01223 348086 at Oncology Clinical Trials Office, Box 193, Addenbrooke's Hospital, Hills Road, Cambridge, CB2 2QQ.

9. Detailed safety study

The first 130 patients randomised are taking part in an ongoing detailed toxicity, dose-intensity and tolerability surveillance study. As well as recording all adverse events, these patients will undergo tests of cardiac and pulmonary function, designed to highlight sub-clinical toxic effects, and thus perhaps alert us to any increased risk of clinically relevant problems becoming apparent later.

These investigations will comprise:

- CXR
- ECG
- Pulmonary function tests: FEV1, FVC, K_{CO}, T_{LCO}
- Echocardiogram/MUGA for LV ejection fraction measurement
- Report any abnormalities or clinically significant acute adverse events during chemotherapy and/or radiotherapy
- Report any Serious Adverse Events (SAEs) as described in section below

The following timepoints:

1. Baseline before chemotherapy
2. Between cycles 4 and 5
3. Immediately after chemotherapy, before radiotherapy
4. 6 months after end of adjuvant chemotherapy
5. In event of any symptomatic deterioration thereafter
6. At 5 years post-treatment
7. At 10 years post-treatment to pick up late toxicity effects

Clinicians need to be aware that anaemia can impact on tests of CO diffusion and every effort should be made to synchronise a FBC with pulmonary function tests. This is particularly important post-treatment when chemotherapy-induced anaemia might provide a falsely low measure of pulmonary diffusion capacity.

Where applicable, all patients will be assessed weekly for acute skin toxicity during radiotherapy according to the gradings listed in Appendix 6. In addition, late skin reactions will be assessed on an annual basis following completion of radiotherapy.

Data from the safety study will be scrutinised carefully by the independent Data & Safety Monitoring Committee after the first 130 patients have completed chemotherapy and radiotherapy.

10. Monitoring of Serious Adverse Events (SAEs)

Investigators must inform the trial unit immediately of any SAEs following the procedures outlined in the section below. Any sporadic cases of possible gemcitabine pulmonary toxicity, should be investigated promptly, and treated according to the guidelines offered in Appendix 3.

Definition

A SAE is any untoward medical occurrence that at any dose:

- Results in death
- Is life-threatening*
- Requires in-patient hospitalisation or prolongation of existing hospitalisation**
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (in offspring of patient regardless of time to diagnosis).

Other important medical events which neither result in death, nor are life threatening, nor require hospitalisation, may be considered serious and adverse if judged to jeopardise the patient and require medical or surgical intervention to prevent one of the outcomes listed above (excluding cancer or result of overdose).

* The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

** Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure, for continued observation. Hospitalisation for a pre-existing condition, including elective procedures, which has not worsened, does not constitute a serious adverse event.

Recording and reporting

In the case of a SAE the Investigator must **immediately**:

- **Fill out** a 'Serious Adverse Event Form'
- **Send** (by fax within 24 hrs of becoming aware of the event) the signed and dated 'Serious Adverse Event Form' to the ***tAnGo*** study team at the CR UK Clinical Trials Unit, Birmingham: **UK Fax: 0800 328 6412; Outside UK Fax: +44 121 414 3700**
- **Telephone** (on day of awareness) the ***tAnGo*** study team at the CR UK Clinical Trials Unit, Birmingham in the case of death or life-threatening events: ☎: **0800 371969 (UK only) or +44(0)121 41 43017 (Mon-Fri, 9 am - 5 pm, UK time**

The ***tAnGo*** study team will inform the Multi-centre Research Ethics Committee (MREC), the Medicines Control Agency and the Medical Departments of the drug manufacturers as appropriate. If requested by MREC, details of SAEs will be sent to all Lead Investigators to forward to their Local Research Ethics Committee (LREC).

Follow-up of SAEs

In the case of a SAE, the subject must be followed-up until clinical recovery is complete and laboratory results have returned to normal, or until disease has stabilised. Follow-up may continue after completion of protocol treatment if necessary. Follow-up information will be noted on the 'Serious Adverse Event Form' by

ticking the box marked 'follow-up' and sending to the study team as information becomes available. Extra annotated information and/or copies of test results may be provided separately. The patient must be identified by trial number, date of birth and initials only.

11. Study organisation

tAnGo will be co-ordinated for the Steering Committee by the Cancer Research UK Clinical Trials Unit in Birmingham, working closely with a number of collaborating clinical trials units in the UK and abroad. This 'hub and spoke' network is intended to help streamline the conduct of the nation's breast cancer trials portfolio by enabling established breast cancer research collaborative groups/ regional trials organisations to work together. It is hoped that this will help to achieve good recruitment and data quality.

11.1 Randomisation of patients

Randomisation should take place within 8 weeks of surgery (but ideally within 1 month). An eligibility form should be completed prior to randomisation. These details should be phoned or faxed through to the ***tAnGo*** study office at the CR UK Clinical Trials Unit or one of the other participating trials units listed below:

Randomisation of patients from centres within UK:

	☎ (9 a.m. – 5 p.m., Mon-Fri)	**Fax (24 hrs)**
CR UK Clinical Trials Unit, Birmingham	0800 371 969 or 0800 731 7625	0800 328 6412
Clinical Trials Research Unit, Leeds	0113 343 4930	0113 343 1471
ISD Cancer Clinical Trials Team, Edinburgh	0131 551 8950	0131 552 4085

Randomisation of patients from centres outside UK:

tAnGo Study Office, Cancer Research UK Clinical Trials Unit, Birmingham

☎: +44 121 414 7844 (9 a.m.-5 p.m., Mon-Fri, UK time) **Fax:** +44 121 414 3700 (24 hrs)

11.2 Forms and data collection

Data collection has been kept to the minimum necessary. Data collected on each subject should be recorded as accurately and completely as possible, and returned promptly:

- Randomisation checklist
- On-study form
- Pathology form & copy of pathology report
- Chemotherapy treatment forms
- Off-study form
- Radiotherapy form (if applicable)
- Pharmacy form
- Quality of Life questionnaires (for participants in Quality of Life study)
- Annual follow-up forms
- Serious Adverse Event form (if applicable)
- Relapse/Death report form (if applicable)

11.3 Protocol compliance and monitoring

tAnGo is being conducted under the auspices of the Cancer Research UK Clinical Trials Unit according to the current guidelines for Good Clinical Practice. Participating centres will be monitored by trials unit staff to confirm compliance with the protocol and the protection of patients' rights as detailed in the Declaration of Helsinki (please refer to section under Ethical Considerations, section 16).

All Clinical Investigators taking part in the trial will be asked to sign a commitment statement and supply a current CV to the trials unit. All clinic personnel should attend a start-up meeting/initiation visit for training on

study procedures and data collection methods. The Lead Investigator must submit this protocol, any supporting documentation, and any subsequent amendments, to their LREC and, if locally required, Institutional Review Boards. Investigators must acquire LREC approval and forward a copy of the written LREC approval letter, signed by the Chairman, to the trials unit before they commence recruitment.

Centres will be visited by a tAnGo monitoring team as required. Study staff will be in regular contact with centre personnel (by phone/fax/email/letter) to check on progress and any queries that they may have. The co-ordinating trials unit will check incoming forms for compliance with the protocol, consistent data, missing data and timing. Investigators will allow the monitors access to source documents as requested. If required the monitoring team will visit centres on a more frequent basis. Centres may be barred from further recruitment in the event of serious and persistent non-compliance and/or very poor recruitment.

Archiving

It is the responsibility of the Principal Investigator to ensure all essential trial documentation and source records (e.g. signed Informed Consent Forms, Investigator Site Files, Pharmacy Files, patients' hospital notes, copies of CRFs etc) at their site are securely retained for at least 5 years after the end of the trial. Do not destroy any documents without prior approval from the CRCTU Document Storage Manager.

11.4 Follow-up

The recommended follow-up scheme below is thought to be appropriate in a higher risk population. This will facilitate accurate and timely capture of relapse events, as well as allowing proper documentation of toxicity and its resolution.

Time from start of treatment	Follow-up appointment / interval
6 months (end of treatment)	3 months
9 months	3 months
12 months	6 months
18 months	6 months
24 months	6 months
30 months	6 months
36 months	6 months
42 months	6 months
48 months	12 months and annually thereafter until 10 years

Annual follow-up data will be collected for 10 years to include sites of recurrence, time of recurrence, subsequent treatment, mortality, and cause of death. Information on second primary breast cancers and other second primary tumours will also be recorded.

Long term follow-up (post 5-years) will be managed by the CRCTU tAnGo Study Office.

11.5 Relapse and death

As soon as definite confirmation has been obtained, a Relapse/Death form should be completed and returned to the trials unit.

***Table 10*: Definition of relapse and death**

Loco-regional	Ipsilateral breast/ chest wall or axillary nodal relapse
Distant	Distant relapse (including supraclavicular nodes)
2nd primary	Including contralateral malignant breast disease
Death	Death from any cause

Relapses will be managed at the discretion of the clinician. A summary of treatment offered at relapse will be requested. This will include any locoregional measures, palliative radiotherapy, and first line metastatic chemotherapy or endocrine regimens. Patients who relapse should remain on follow-up.

12. Quality of Life sub-study

A sub-study addressing quality of life will be assessed in a cohort of 500 patients. Collaboration with this part of the trial is optional for centres. All patients randomised from participating centres will be invited to take part in the sub-study. This sub-study will use the EORTC QLQ-C30 (version 3) (with the additional Breast Cancer module EORTC QLQ-BR23), the Women's Health Questionnaire, and the EQ-5D Health Questionnaire. Patients will also be asked to complete diary sheets during their treatment. Quality of life information will be collected before chemotherapy starts, at mid-point, at the end of chemotherapy, and then at 6 and 18 months after the end of treatment.

13. Statistical considerations

The detailed safety study will be a clinical, pulmonary function, and radiological assessment of 130 patients intended to provide early notification of any increase in the risk of pulmonary toxicity attributable to gemcitabine/paclitaxel/radiation combination. A previous retrospective analysis has indicated a 1.6% risk of pulmonary toxicity associated with single agent gemcitabine based on the incidence of symptomatically-driven diagnoses [66], and we expect a similar level of risk in this study. Thus, recruiting 65 patients into each treatment arm in the safety study would allow detection of a 10-fold difference (i.e. 1.5 versus 15% or 2 versus 20%) in the risk of pulmonary toxicity relative to this, with an 80% power at the 5% level of significance (2-sided). Note that, unlike the earlier retrospective analyses, this safety study will not rely on symptom-driven diagnosis, but will also involve acute, quantitative detection of sub-clinical disturbances in pulmonary function, as revealed by FEV1/FVC measurement, and carbon monoxide diffusion tests. Also, records of adverse events in the study population as a whole will enable detection of more modestly increased levels of risk.

The primary endpoint for this study is disease-free survival. We estimate that the 5-year disease-free survival rate for patients treated with EC-paclitaxel in this higher risk group will be approximately 70%. Thus, recruiting 1500 patients (450 events expected after 5 years follow up) into each treatment arm will allow absolute differences in survival rates in excess of 5% to be detected (e.g. 70% to 75%) at the 5% (2-sided) level of significance with 85% power. This would also allow differences in excess of 7% (e.g. 70% to 77%) to be detected with 99% power, and in excess of 10% (e.g. 70% to 80%) with 99.9% power. Hence, the trial aims to recruit a total of 3000 women, in order to detect realistic but important differences. This is based on an assumption that 15-20% of patients recruited will be high-risk node negatives and the remaining 80-85% node-positives.

Randomisation will be stratified by country (of randomising hospital) (England; Scotland; Wales; any other countries as they join), nodal status (negative; 1 to 3; 4 or more nodes involved), ER status (negative; weakly positive; positive), age (≤50; >50 years), radiotherapy (no; yes) and HER2 status (+++; other; not measured) to avoid imbalance in the two treatment arms. Baseline prognostic information on number of nodes involved, grade, PgR status and tumour size will be recorded for use in the analysis.

13.1 Analysis plan

Overall survival will be calculated from the date of randomisation to the date of death, or to the censor date. The analysis of disease-free survival will be calculated from the date of randomisation to the date of first relapse or death, if no date of relapse is recorded, or the censor date. Analysis will be carried out on all cause mortality and disease-free survival using the log-rank analysis on an intention-to-treat basis. Treatment comparisons will be tested, using Cox regression modelling and hazard ratio plots, with and without adjustment for the stratification and baseline prognostic factors of nodal status, age, grade, tumour size, radiotherapy, ER, PgR and HER2 status, and menopausal status.

The first data monitoring analysis will be performed when approximately 500 patients have been recruited which should correspond to the detailed acute toxicity and safety being available on the first 130 patients to have completed both chemotherapy and radiotherapy in the safety study. There are three planned log-rank analyses of the primary endpoint; the first with a minimum of 18 months follow-up (i.e. when approximately 280 events have been reported) which will allow the detection of 10% differences in absolute survival rates with a 95% power at the 5% level (2-sided), the second at a minimum of 30 months follow-up (i.e. when

approximately 550 events have been reported) which will allow the detection of 7% differences in absolute survival rates with a 95% power at the 5% level (2-sided) and the third at a minimum of 60 months follow-up (i.e. when approximately 920 events have been reported) which will allow the detection of 5% differences with a 90% power at the 5% level (2-sided).
These analyses will be presented to the Data & Safety Monitoring Committee but will not be publicly reported until recruitment has closed or unless the Data & Safety Monitoring Committee recommends it. At the first presentation of results data on treatment toxicity and health care economics will be available.

13.2 Independent Data and Safety Monitoring Committee (DSMC)

The analyses will be supplied to an independent DSMC, which will be asked to give advice on whether the accumulated data from this trial, together with the results from other relevant trials, justifies the continuing recruitment of further patients. This committee will meet annually; the exact frequency will depend on the rate of accrual and event rates. The DSMC will monitor recruitment to the trial and protocol compliance as well as toxicity and serious adverse events. The main outcomes will be analysed as stated above in the analysis plan.

There are three planned analyses of the primary endpoint of disease free survival, which equate to the minimum follow-up of 18, 30 and 60 months. Given the evidence from the adjuvant breast cancer setting provided by *Berry (2004) and **Hudis (2005), it is assumed that the majority of events will happen in the first 3 years of follow-up. This trend has been observed in the NEAT adjuvant breast cancer trial as well as the CALGB 8495, 9344 and 9741 trials. These analyses of the primary outcome will be reported to the DSMC adjusting for the three planned looks at the data using Pocock's method of assigning equal weighting to the alpha spend with significance determined by p=0.022; (Pocock, S.J., (1982) Interim analysis for randomized clinical trials: the group sequential approach. Biometrics, **38**: 153-62). The DSMC will also assess the overall progress of the trial and evaluate world-wide evidence when monitoring the trial. Recruitment will also be scrutinised by the Steering Committee at least 6-monthly intervals as well as the annual reports from the DSMC.

*Berry, D. A (2004) General Session 4: Effects of improvements in chemotherapy on disease-free survival and overall survival of estrogen-receptor negative, node-positive breast cancer: 20-year experience of CALGB & U.S. Breast Intergroup. Presented at 2004 27th San Antonio Breast Cancer Symposium. http://www.abstracts2view.com/sabcs/sessionindex.php?p=2

**Hudis, C (2005) General Session 7: Five year follow-up of INT C9741: dose-dense (DD) chemotherapy (CRx) is safe and effective. Presented at 28th San Antonio Breast Cancer Symposium. http://www.abstracts2view.com/sabcs05/view.php?nu=SABCS05L_372

13.3 Milestones

tAnGo will randomise 3000 patients, primarily from an estimated 100 centres in the UK. The aim is to complete accrual within 3 years if possible by maximising the number of UK centres and the speed with which they are activated. In order to complete accrual rapidly, recruitment will be opened to interested European centres. Participation by North American and other international centres will be welcomed.

The planned safety sub-study began recruitment in August 2001; the 130th patient was enrolled in October 2002. The main body of the trial was formally launched in January 2003. The following milestones assume projected event rates which may alter depending on the patient populations recruited.

- Aug 2001: Start randomisation
- Oct 2003: Last safety sub-study patient completes post-radiotherapy cardiac and pulmonary function tests
- Dec 2003: Present analysis of first 130 patient safety study and report on available data from main study at first DSMC meeting
- Dec 2003: 1000 patients recruited
- Nov 2004: Finish recruitment of 3000 patients.
- Mid-2006: First planned analysis of interim data with a minimum of 18 months follow-up (approximately 280 events have occurred). Presentation of results including toxicity, dose-intensity and Quality of Life study.

- Mid-2007 Second analysis of interim data with a minimum of 30 months follow-up (approximately 550 events have occurred).
- Mid-2008 Last safety sub-study patient completes post-radiotherapy, 5 year cardiac and pulmonary function tests
- End-2011 5-year analysis of primary endpoints assuming all patients followed-up for at least 5 years (approximately 920 events).
- Mid-2013 Last safety sub-study patient completes post-radiotherapy, 10 year cardiac and pulmonary function tests
- End-2015 Analysis of long-term follow-up and events with a minimum follow-up of 10 years.

14. Concurrent studies

Patients recruited to the ***tAnGo*** trial will be ineligible for intercalated/sandwiched radiotherapy, and thus excluded from the *SECRAB* trial.

In the experience of the CR UK Clinical Trials Unit, if appropriately supported, patients can be successfully randomised into multiple studies and, accordingly, ***tAnGo*** patients may be offered the opportunity to participate in other appropriate randomised studies, providing these have been approved by the Steering Committee. All investigators will be kept up to date with information about ongoing candidate national, peer-reviewed studies.

14.1 Herceptin® maintenance trials

Assuming they are otherwise eligible, patients randomised into ***tAnGo*** will remain candidates for the BIG-co-ordinated Herceptin™ maintenance trial (HERA/BIG 01-01), addressing the role of trastuzumab in patients whose tumours over-express HER2. Randomisation into HERA will be encouraged, and details of HER2 status will be requested as a stratification criterion at the point of randomisation into ***tAnGo*** in order to control for any bias caused by entry into HERA. However, this stratification will accommodate centres in which determination of HER2 status is not presently routine (see Trial Schema, section 2).

14.2 AZURE trial

Patients entered into ***tAnGo*** may be offered randomisation within the AZURE study, a phase III randomised trial addressing the role of bisphosphonates (zoledronic acid, Zometa®) in adjuvant therapy for early stage breast cancer. Although we will not be stratifying patients by AZURE participation or not, we will capture this information from the Off Study form.

14.3 OPTION trial

Appropriate ***tAnGo*** patients may be offered randomisation within the OPTION study, a phase III randomised trial addressing the benefits of temporary ovarian suppression during chemotherapy on subsequent fertility and preservation of ovarian function in the longer term in pre-menopausal women.

15. Financial matters

tAnGo is an Investigator-led and -designed trial which is funded through educational grants from the pharmaceutical industry and Breast Cancer Relief. The study has been independently peer reviewed (gaining high alpha) and endorsed by the Cancer Research Campaign, and meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England.

Free gemcitabine (Gemzar®) will be made available throughout. Paclitaxel (Taxol®) will be provided free of charge in both arms of the study up to UK licence. After licence, paclitaxel will be provided at 50% cost price in both arms up to NICE endorsement, and at cost price in both arms thereafter.

15.1 Indemnity

tAnGo is co-ordinated by the CR UK Clinical Trials Unit in Birmingham. The Trials Unit does not hold insurance against claims for compensation for injury caused by participation in a clinical trial and they cannot offer any indemnity. As this is a clinician-initiated study, ABPI guidelines for patient compensation by the pharmaceutical industry will not apply. However, in terms of liability, NHS Trust and Non-Trust Hospitals have a duty of care to patients treated, whether or not the patient is taking part in a clinical trial. Compensation is therefore available in the event of injury through clinical negligence being proven.

16. Ethical and regulatory standards

16.1 Ethical considerations

This study will be carried out in accordance with the World Medical Association (WMA) Declaration of Helsinki (1964) and the Tokyo (1975), Venice (1983), Hong Kong (1989), South Africa (1996) and Scotland (2000) amendments. Copies of the Declaration may be obtained by contacting the trials unit, or from the WMA website: http://www.wma.net/e/policy/17-c_e.html. The Office for Protection from Research Risks (OPRR) awarded an International Co-operative Project Assurance (ICPA) to the CR UK Clinical Trials Unit in May 1999 enabling the unit to conduct Co-operative Group protocols in the USA.

The protocol has gained MREC approval. Before entering patients into the study, the responsible Investigator must ensure that the protocol has the approval of the relevant LREC.

16.2 Informed consent

It is the responsibility of the Investigator to obtain written informed consent in compliance with national requirements from each patient prior to entering the trial or, where relevant, prior to evaluating the patient's suitability for the study.

16.3 Patient confidentiality

The personal data recorded on all documents will be regarded as strictly confidential. To preserve the patient's anonymity, only their initials, date of birth, and hospital number will be recorded on the case report forms. With the patient's permission, their name will be collected at randomisation to allow flagging with the Office of National Statistics, The Investigator must ensure the patient's anonymity is maintained. The Investigator must maintain documents not for submission to the trials unit (e.g. patients' written consent forms) in strict confidence.

The Trials Unit will maintain the confidentiality of all patient data and will not reproduce or disclose any information by which patients could be identified. Patients should be reassured that their confidentiality will be respected at all times.

In the case of special problems and/or governmental queries, it will be necessary to have access to the complete study records, provided that patient confidentiality is protected.

16.4 Patient withdrawal

Patients have the right to withdraw from the study at any time for any reason. The Investigator also has the right to withdraw patients from the study. Full details of the reasons for withdrawal should be recorded on the Off-study form if clinician-initiated, otherwise a simple statement reflecting patient preference will suffice. Withdrawn patients should be followed-up in accordance with the protocol.

16.5 Annual report

The ***tAnGo*** study office will send an annual trial update report to the MREC which will be forwarded to each participating centre, together with details of their individual recruitment. It will be the responsibility of each participating centre to send a copy of this report onto their LREC.

16.6 End of trial and archiving

The trial end date is deemed to be the date of last data capture following 10 years of follow-up.
The competent authority and the REC will be provided with a summary of the clinical trial report within 12 months of the end of the trial.

The archiving period will begin immediately after this date. All essential trial documents (including patient notes) must be retained for at least 5 years following the end of the trial. The trial sponsor will notify the centres when documents may be destroyed.

17. Publication policy

The main trial results will be published in the name of the trial in a peer-reviewed journal, on behalf of all collaborators. The manuscript will be prepared by a writing group, appointed from amongst the Steering Committee, representatives of the regional trials groups, and high accruing Investigators. The trials unit and all participating centres and Investigators will be acknowledged in this publication. All presentations and publications relating to the trial must be authorised by the ***tAnGo*** Steering Committee.

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Appendix 1: Cyclophosphamide

Mode of action

Cyclophosphamide is an oxazophorine pro-drug. It undergoes activation to phosphoramide mustard, an alkylating agent which acts by nucleophilic substitution to form a covalent bond between a nucleophilic DNA site (centre with an unshared electron), such as the N^7 or O^6 position of guanine, and the electrophilic (electron-poor, positively charged carbon) of its own alkyl group.

Toxicity

Myelosuppression: leucopenia is the major dose-limiting toxicity. Thrombocytopenia and anaemia are less prominent. These effects are usually reversible. Immuno-suppressive and anti-inflammatory actions may be associated with cyclophosphamide use.

Cardiac Toxicity: cardiac toxicity has been observed with high dose cyclophosphamide (120 to 270 mg/kg). On occasion, severe and sometimes fatal, congestive heart failure has occurred within a few days of first dose, and histopathological examination then primarily shows haemorrhagic myocarditis. This side effect was not observed prior to the introduction of high dose therapy, and has not been linked with standard dose therapy. No residual cardiac abnormalities (as evidenced by abnormalities of ECG or echocardiogram) are seen in patients surviving episodes of cardiac toxicity associated with high doses of cyclophosphamide. Cyclophosphamide may potentiate anthracycline-induced cardiotoxicity.

Carcinogenesis: a variety of second malignancies may occur in patients treated with cyclophosphamide. These include bladder, myeloproliferative and lymphoproliferative malignancies. They are most frequently observed in patients treated for primary myeloproliferative or lymphoproliferative malignancies or non-malignant disease in which immune processes are believed to be involved pathologically. In some cases, the second malignancy developed several years after cyclophosphamide treatment had been discontinued. Urinary bladder malignancies generally occur in patients with previous haemorrhagic cystitis.

Impaired fertility: temporary infertility is common. Prospects of permanent infertility increase with age.

Foetal damage and teratogenicity: cyclophosphamide is teratogenic and causes foetal resorption in experimental animals. It should not be used in pregnancy, particularly in early pregnancy, unless the potential benefits outweigh the possible risks. Secure methods of contraception are required.

Lactation: cyclophosphamide is excreted in breast milk and breast-feeding should be terminated prior to institution of cyclophosphamide therapy.

Gastrointestinal: anorexia, nausea, and vomiting are common and related to dose as well as individual susceptibility. There are isolated reports of haemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy.

Hair and skin toxicity: temporary alopecia is frequent. Regrowth of hair can be expected although occasionally the new hair may be of a different colour or texture. Skin and fingernails may darken during therapy. Non-specific dermatitis has been reported.

Urothelial: sterile haemorrhagic cystitis may be caused by acrolein urinary metabolites. Non-haemorrhagic cystitis and/or bladder fibrosis are also reported. Atypical epithelial cells may be found in the urinary sediment. Ample fluid intake and frequent voiding are beneficial. Haematuria usually resolves spontaneously within a few days but may persist for several months. In severe cases, transfusion may be required, as may electrocautery to telangiectatic areas of the bladder, and diversion of urine flow. Nephrotoxicity, caused by haemorrhage and clot formation in the renal pelvis, or haemorrhagic ureteritis are unusual. Once such problems have occurred, the urothelial protective agent Mesna may be used prophylactically.

Pulmonary toxicity: interstitial pulmonary fibrosis has been reported in a few patients receiving high doses of cyclophosphamide over a prolonged period. Pneumonitis is also possible. Symptoms include dyspnoea, dry cough and fever. The signs include basal crackles. The typical radiological appearance is a fine reticulo-nodular pattern. Steroids may promote recovery. Cyclophosphamide-induced pneumonitis may otherwise

take one or more months to resolve, following completion of therapy. However, onset may be delayed from 2 months to 8 years following discontinuation of treatment. Pulmonary problems are rare and sporadic.

Other adverse reactions: these may include headache, dizziness, hypoprothrombinaemia and diabetes mellitus. Also, the possibility of anaphylactic reaction to cyclophosphamide should not be excluded.

Phamacokinetics

The plasma half-life of the unchanged drug is apparently independent of age, nationality, sensitivity or resistance to the drug, diagnosis, or dosage. In patients who have received no drug therapy known to influence microsomal metabolic rates, the average half-life of unchanged cyclophosphamide is between 5.0 and 6.5 hRs after i.v. administration of C^{14}-labeled cyclophosphamide.

Distribution: the drug and its metabolites are distributed throughout the body. Following i.v. administration of cyclophosphamide, both unchanged drug and metabolites pass the blood-brain barrier. Cerebral tissue has been shown to contain drug levels in a concentration range similar to that found in blood. Biopsies performed 2 hrs after administration of the drug reveal about 30% more drug to be present in lymph nodes than in muscle, adipose tissue, or skin, but the relative proportion of unchanged drug metabolites has not been established.

Cyclophosphamide does not bind to human plasma proteins in appreciable amounts, but with single i.v. doses about 12 - 14% of the total dose was bound to plasma proteins at plasma cyclophosphamide concentrations of 10 - 200 µmoles/mL. Repeated doses increased the amount bound to plasma proteins. Following 5 doses of 40 mg/kg, about 56% of the dose was bound.

Metabolism: cyclophosphamide is metabolically activated in the liver by the cytochrome P-45-mixed-function oxidase system of the smooth endoplasmic reticulum. This converts cyclophosphamide to 4-hydroxycyclophosphamide, which exists in a steady state with the acyclic tautomer, aldophosphamide.

Cyclophosphamide is itself biologically inactive, but is eliminated from the body only very slowly. The activated metabolites either alkylate target sites in susceptible cells in an all-or-none type of reaction, or are degraded by formation of inactive metabolites and rapidly excreted by the kidneys.

Peak plasma concentrations of metabolites have been found to be almost proportional to the administered dose, but there is considerable inter-individual variability in the rate of metabolism of cyclophosphamide. Peak plasma alkylating metabolite levels generally are reached at 2 - 3 hours after administration of the drug. The average plasma alkylating metabolite concentration at 8 hours after i.v. administration of the drug was about 77% of the peak level when studied in 12 patients without prior drug exposure.

Inverse relationships have been described between the measured area under the plasma concentration-time curve (AUC) of the parental compound and response. Response has been shown to relate directly to increase in concentration or AUC of active metabolites.

Excretion: a generally higher proportion of the administered dose is excreted in the urine as metabolites, than parent drug. Recovery of radioactivity after i.v. administered labelled cyclophosphamide ranged from 37-82%, with 20- 45% of that recovered attributable to the unchanged drug. The total urinary excretion of unchanged cyclophosphamide ranged from 3- 30% of the dose with most cases in the upper half of the range.

Precautions

Renal impairment: caution is required in patients with impaired renal function. Dose reductions of 50% are recommended if the GFR drops below 20 mL/min.

Anaesthesia: since cyclophosphamide is an inhibitor of serum cholinesterase, patients receiving the drug may exhibit an increased sensitivity to neuromuscular blocking agents such as succinylcholine. If a patient receiving cyclophosphamide is to undergo surgery, the anaesthetist should therefore be advised.

Contraindications: Sensitivity to cyclophosphamide or to any components of its dosage forms, severe leukopoenia, thrombocytopoenia, severe hepatic dysfunction.

Appendix 2: Epirubicin

Mode of action

Epirubicin (Pharmorubicin®; Ellence®) is an anthracycline cytotoxic agent. Although it is known that anthracyclines can interfere with a number of biochemical and biological functions within eukaryotic cells, the precise mechanisms underlying their cytotoxic and/or antiproliferative properties have yet to be elucidated.

Epirubicin forms a complex with DNA by intercalation of its planar rings between nucleotide base pairs, with consequent inhibition of nucleic acid (DNA and RNA) and protein synthesis. Intercalation triggers DNA cleavage by topoisomerase II, resulting in cytocidal activity. Epirubicin also inhibits DNA helicase activity, preventing the enzymatic separation of double-stranded DNA and interfering with replication and transcription. Epirubicin is also involved in oxidation/reduction reactions by generating cytotoxic free radicals. The antiproliferative and cytotoxic activity of epirubicin is thought to result from these or other possible mechanisms.

Toxicity

Myelosuppression: dose-dependent, reversible leucopoenia and/or neutropenia days 10-14 predominates and represents the most common acute dose-limiting toxicity. In most cases leucopoenia/neutropenia is usually transient, with recovery to normal values within 21 days of drug administration. Severe thrombocytopenia and anaemia can also occur, especially in combination. Clinical consequences may include fever, infection, septicaemia, septic shock, haemorrhage, tissue hypoxia, symptomatic anaemia, or death. Careful monitoring is required, together with appropriate supportive measures (e.g., intravenous antibiotics, colony-stimulating factors, transfusions) where necessary.

Cardiac Function: Cardiotoxicity is a well documented risk of anthracycline treatment, and be manifest by early (or acute) or late (delayed) events. Early cardiac toxicity consists mainly of sinus tachycardia and/or ECG abnormalities such as non-specific ST-T wave changes. However, tachyarrhythmias, including premature ventricular contractions and ventricular tachycardia, bradycardia, as well as atrioventricular and bundle-branch block have also been reported. These effects do not usually predict subsequent development of delayed cardiotoxicity, are rarely of clinical importance, and are generally not considered an indication for the suspension of epirubicin treatment.

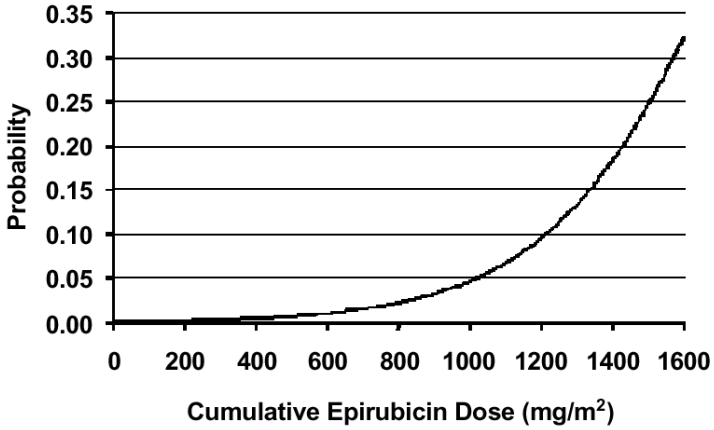
Delayed or cumulative cardiac toxicity results from a characteristic cardiomyopathy characterised by reduced LVEF and/or congestive heart failure (CHF), sometimes life-threatening. Cardiac toxicity represents the cumulative dose-limiting toxicity of the drug and usually develops late in the course of therapy, or within 2 - 3 months of completion. However, CHF has been reported months to years after exposure.

In a retrospective survey, involving 9144 patients, most with advanced stage solid tumours, the probability of developing CHF increased from 0.9% at a cumulative dose of 550 mg/m^2, to 1.6% at 700 mg/m^2, and to 3.3% at 900 mg/m^2(see Figure A1). The risk of developing CHF in the absence of other cardiac risk factors increased steeply after an epirubicin cumulative dose of 900 mg/m^2. In another retrospective survey of 469 epirubicin-treated patients with metastatic or early breast cancer, the reported risk of CHF was comparable to that observed in the larger study of over 9000 patients. Prospectively acquired data from the epirubicin adjuvant studies submitted for US approval are shown in Table A1.

***Table A1*: Incidence of delayed cardiac adverse events in MA-5 and GFEA-05 trials [14,15].**

	Percent of patients suffering cardiac toxicity by adjuvant regimen		
Event / Regimen	**FEC-100/FEC-120 (n=620)**	**FEC-50 (n=280)**	**CMF (n=360)**
Asymptomatic reduction in LVEF	**1.8%**	**1.4%**	**0.8%**
Congestive cardiac failure	**1.5%**	**0.4%**	**0.3%**

Fig A1. **Increasing risk of cardiac failure with cumulative epirubicin dose**



A cumulative dose of 900 mg/m^2 epirubicin should be exceeded with subsequent therapy at relapse, only with extreme caution. Other factors, including active or dormant cardiovascular disease, prior radiotherapy to the mediastinal/pericardial area, previous therapy with anthracenediones, or concomitant use of other negative ionotropes may increase the risk of cardiac toxicity at lower epirubicin doses.

Although endomyocardial biopsy is recognised as the most sensitive diagnostic tool to detect anthracycline-induced cardiomyopathy, this is not performed routinely. ECG changes such as dysrhythmias, reduced QRS voltage, or prolongation of systolic time interval may be indicative of anthracycline-induced cardiomyopathy, but ECG is neither sensitive nor specific as a means of detecting, or monitoring anthracycline-related cardiotoxicity. In the event of underlying cardiac risk factors, the risk of serious cardiac decompensation may be minimised by regular monitoring LVEF during epirubicin treatment, with prompt withdrawal at the first sign of impaired function. The preferred method is multi-gated radionuclide angiography (MUGA) or echocardiography. A baseline cardiac evaluation with ECG and MUGA scan, or an echocardiogram, is recommended in patients with risk factors for increased cardiac toxicity. Repeated MUGA or echocardiographic determinations of LVEF should be performed, particularly with higher, cumulative anthracycline doses. The technique used for assessment should be consistent through follow-up.

Mutagenicity and carcinogenicity: epirubicin is mutagenic to bacteria *in vitro* (Ames test), and to a variety of mammalian cell lines (HGPRT). Epirubicin is also mutagenic and carcinogenic in animals. Epirubicin is clastogenic *in vitro* (causing chromosome aberrations in human lymphocytes). Secondary acute myelogenous leukaemia (AML), with or without a pre-leukaemic phase, has been reported in patients previously treated with anthracyclines. Secondary leukaemia is more common when given in combination with DNA-damaging anti-neoplastic agents, when patients have been heavily pre-treated with cytotoxic drugs, or when doses of the anthracyclines have been escalated. These leukaemias can have a short 1- to 3-year latency period. An analysis of 3844 patients who received adjuvant treatment with epirubicin in several controlled clinical trials showed a cumulative risk of secondary AML of about 0.2% (approximate 95% C.I., 0.05-0.4) at 3 years and approximately 0.8% (approximate 95% C.I., 0.3-1.2) at 5 years. A similar incidence of leukaemia has been encountered in the CALGB 9344 trial, with doxorubicin; four patients have developed leukaemia to date after AC, and 2 after AC-paclitaxel. To date, with a median follow-up of 34 months, secondary AML, or myelodysplastic syndrome, has been reported in five patients (0.16%) in NSABP-B28.

Impaired fertility: epirubicin may cause temporary or irreversible amenorrhoea (premature menopause) in pre-menopausal women.

Foetal damage and teratogenicity: embryotoxicity (increased late resorptions, post-implantation losses, retarded foetal growth and dead foetuses; and fewer live foetuses), decreased placental weight and teratogenicity have been observed in rats. Teratogenic defects included numerous external (anal atresia, mis-shapen tail, abnormal genital tubercle), visceral (primarily gastrointestinal, urinary, and cardiovascular systems), and skeletal (deformed long bones and girdles, rib abnormalities, irregular spinal ossification) malformations. There are no adequate and well-controlled studies in pregnant women. Two pregnancies

have been reported in women taking epirubicin. A 34-year-old woman, 28 weeks pregnant at the time of her diagnosis of breast cancer, was treated with cyclophosphamide and epirubicin every 3 weeks for three cycles. She received the last dose at 34 weeks of pregnancy and delivered a healthy baby at 35 weeks. A second 34-year-old woman with breast cancer metastatic to the liver was randomised to FEC-50 but was removed from study because of pregnancy. She experienced a spontaneous abortion. If epirubicin is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the foetus. Women of childbearing potential should be advised to avoid pregnancy.

Lactation: epirubicin is excreted into the milk of rats when given peri- and post-natally. It is not known whether epirubicin is secreted in human milk. Because other anthracyclines are excreted in human milk and because of the potential for serious adverse reactions in nursing infants, mothers should discontinue nursing prior to commencing epirubicin.

Thrombophlebitis: venous sclerosis may result from epirubicin injection into a small vessel or from repeated injections into the same vein.

Extravasation: extravasation during the infusion may cause local pain, severe tissue inflammation and necrosis. Epirubicin should be administered slowly into the side-arm of a free-running saline i.v. infusion. Veins over joints or in extremities with compromised venous or lymphatic drainage should be avoided if possible. The dose should be administered over 3 - 5 min. A burning or stinging sensation may be indicative of peri-venous infiltration, and the infusion should be immediately terminated and restarted in another vein. However, peri-venous infiltration may occur without causing pain.

Facial flushing/ local erythematous streaking along the vein: may be indicative of excessively rapid administration. It may precede local phlebitis or thrombophlebitis.

Gastrointestinal: epirubicin is emetic. Diarrhoea and abdominal pain are rare.

Mucositis: dose-dependent oral stomatitis and, less often oesophagitis may occur. Clinical manifestations include a burning pain, erythema, erosions, ulcerations, bleeding, or infections. If severe, mucositis may progress to mucosal ulceration, but recovery is usual within 3 weeks of therapy. Hyperpigmentation of the oral mucosa may also occur.

Conjunctivitis/scleritis: this affected 14.8% of 620 patients treated with FEC-100, or FEC-120, but was exclusively grade 1-2. It is best treated with simple measures.

Hair and skin: alopecia is near inevitable. Photosensitisation is common, and requires precautions.

Pharmacokinetics

Epirubicin pharmacokinetics are linear over the dose range of 60 - 150 mg/m^2. Plasma clearance is not affected by the duration of infusion or administration schedule. The plasma concentration declines in a triphasic manner with mean half-lives for the alpha, beta, and gamma phases of about 3 min, 2.5 hrs and 33 hrs, respectively.

Distribution: Following intravenous administration, epirubicin is rapidly and widely distributed into the tissues. Plasma protein binding is about 77% and is not affected by drug concentration. Epirubicin concentrates in red blood cells; whole blood concentrations are approximately twice those of plasma.

Metabolism: Epirubicin is extensively and rapidly metabolised by the liver and also by other organs and cells, including red blood cells. Four main metabolic routes have been identified: (1) reduction of the C-13 keto-group with the formation of the 13(S)-dihydro derivative, epirubicinol; (2) conjugation of both the unchanged drug and epirubicinol with glucuronic acid; (3) loss of the amino sugar moiety through a hydrolytic process with the formation of the doxorubicin and doxorubicinol aglycones; and (4) loss of the amino sugar moiety through a redox process with the formation of the 7-deoxy- doxorubicin aglycone and 7-deoxy-doxorubicinol aglycone. Epirubicinol has *in vitro* cytotoxic activity one-tenth that of epirubicin. As plasma levels of epirubicinol are lower than those of the unchanged drug, they are unlikely to reach *in vivo* concentrations sufficient for cytotoxicity. No significant activity or toxicity has been reported for the other metabolites.

Excretion: Epirubicin and its major metabolites are eliminated in bile and, to a lesser extent, in urine. Mass-balance data from one patient found about 34% of the total radioactive dose in faeces, and 27% in urine. These data are consistent with those from patients with extrahepatic obstruction and percutaneous drainage, in whom approximately 35% and 20% of the administered dose were recovered as epirubicin or its major metabolites in bile and urine, respectively, in the 4 days after treatment.

Precautions

Age: A population analysis of plasma data from 36 cancer patients (13 males and 23 females, 20 - 73 years) showed that age affects plasma clearance of epirubicin in female patients. The predicted plasma clearance for a female patient of 70 years of age was about 35% lower than that for a female patient of 25 years of age. Although a lower epirubicin starting dose does not appear necessary in elderly female patients, and has not been used in clinical trials, particular care should be taken in monitoring toxicity when epirubicin is administered to female patients >70 years of age.

Hepatic Impairment: epirubicin is eliminated by both hepatic metabolism and biliary excretion and clearance is reduced in patients with hepatic dysfunction. In a study of the effect of hepatic dysfunction, patients with solid tumours were classified into three groups. Patients in Group 1 (n=22) had serum AST (SGOT) levels above the upper limit of normal (median: 93 IU/L) and normal serum bilirubin levels (median: 0.5 mg/dL) and were given epirubicin doses of 12.5 to 90 mg/m^2. Patients in Group 2 had alterations in both serum AST (median: 175 IU/L) and bilirubin levels (median: 2.7 mg/dL) and were treated with an epirubicin dose of 25 mg/m^2 (n=8). Their pharmacokinetics were compared to those of patients with normal serum AST and bilirubin values, who received epirubicin doses of 12.5 to 120 mg/m^2 . The median plasma clearance of epirubicin was decreased compared to patients with normal hepatic function by about 30% in patients in Group 1 and by 50% in patients in Group 2. Patients with more severe hepatic impairment have not been evaluated.

Definitive recommendation regarding use of epirubicin in patients with hepatic dysfunction are not available. Patients with liver dysfunction at the outset are excluded from this protocol. However, in the event of problems developing on study, the following dose reductions are recommended:

- Bilirubin 1.2 to 3 mg/dL or AST 2-4 × upper limit of normal: 50% standard dose.
- Bilirubin > 3 mg/dL or AST >4 × upper limit of normal: 25% standard dose.

Renal Impairment: No significant alterations in the pharmacokinetics of epirubicin or its major metabolite, epirubicinol, have been observed in patients with serum creatinine <5 mg/dL. A 50% reduction in plasma clearance was reported in four patients with serum creatinine >5 mg/dL Patients on dialysis have not been studied. While no specific dose recommendation can be made based on the limited available data in patients with renal impairment, lower doses should be considered in patients with severe renal impairment (serum creatinine >5 mg/dL).

Drug-drug interactions

Cimetidine: co-administration of cimetidine (400 mg twice daily for 7 days starting 5 days before chemotherapy) increased the mean AUC of epirubicin (100 mg/m^2) by 50% and decreased its plasma clearance by 30%.

Drugs metabolised by cytochrome P-450 enzymes: no systematic *in vitro* or *in vivo* evaluation has been performed to examine the potential for inhibition or induction by epirubicin of oxidative cytochrome P-450 isoenzymes.

Calcium channel blockers: concomitant use of cardio-depressant compounds such as calcium channel blockers requires close monitoring of cardiac function throughout treatment.

Appendix 3: Gemcitabine

Introduction

Gemcitabine (2'deoxy-2'.2'-difluorocytidine monohydrochloride (dFdC, β isomer)) was initially synthesised as a potential antiviral drug with excellent activity against both RNA and DNA viruses *in vitro*. Its poor therapeutic index (in this setting) and its characterisation as a potent and specific deoxycitidine analogue prompted its subsequent successful evaluation as a cytotoxic. After showing broad spectrum activity in a range of pre-clinical models, gemcitabine entered phase I clinical trials in 1987 using a day 1, day 8, and day 15, q 28 day regimen, and a 30 min infusion. Gemcitabine (Gemzar®) is licensed for use against non-small cell lung cancer and pancreatic cancer, and has been shown to improve quality of life.

Mode of action

Like other antimetabolites, gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and blocking the progression of cells through the G1/S-phase boundary. As a pro-drug, it is phosphorylated by deoxycitidine kinase to dFdC-5'-monophosphate (dFdCMP), then to dFdC5'-diphosphate (dFdCDP), and subsequently dFdC-5'triphoshate (dFdCTP), the two identified active moieties. dFdCDP inhibits ribonucleotide reductase which is responsible for catalysing the reactions that generate the deoxynucleoside triphosphates required for DNA synthesis. Inhibition of this enzyme causes a reduction in the intracellular concentrations of deoxynucleotides, including dCTP, which enhances the competitive incorporation of gemcitabine triphosphate into DNA (self-potentiation). When incorporated, dFdCTP results in premature chain termination. After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain termination). In comparison with ara-CTP, dFdCTP is less readily excised by DNA exonucleases, and this may account for its better intracellular accumulation and different spectrum of clinical activity. In CEM T lymphoblastoid cells, gemcitabine induces internucleosomal DNA fragmentation, one of the characteristics of programmed cell death.

Toxicity

Myelosuppression: short-lived thrombocytopenia is dose-limiting. Neutropenia is less pronounced. Phase I studies using a day 1, day 8, day 15, q 28 day, 30 min infusions have defined MTDs in the range 790 – 1375 mg/m^2. Longer infusions are more myelotoxic. In pancreatic studies red blood cell transfusions were required by 19% of patients, and the incidence of sepsis was less than 1%. Note that gemcitabine may cause fever in the absence of clinical infection, most usually associated with flu-like symptoms (see below).

Cardiotoxicity: typically, 2% of patients treated with single agent gemcitabine have discontinued treatment for myocardial infarction, cerebrovascular accident, arrhythmia, or hypertension, but many of these had prior cardiovascular disease.

Pulmonary toxicity: sporadic cases of severe pulmonary toxicity (pneumonitis) have been reported. The mechanism underlying this problem is presently unknown. However, the incidence of radiological pulmonary infiltrates is less than 1%, and the literature reports only one attributable death. A recent review of patients entered in the Eli Lilly and Company Limited clinical trial database of 4448 patients, confirmed 32 patients with serious pulmonary toxicity, (0.72%). Of these 32, just 12 patients (0.27% of total) suffered dyspnoea. Of a further 296 cases of possible pulmonary toxicity reported in 217,400 patients estimated to have been treated off study world wide, 167 (0.077%) were thought to have suffered serious pulmonary toxicity attributable to gemcitabine. The incidence of dyspnoea was 0.02%. In 11 of these incidents there was concomitant treatment with paclitaxel or mitomycin, both of which have been individually associated with occasional (rare) episodes of pneumonitis.

In another retrospective analysis of 314 patients treated at the Fox Chase Cancer Centre 1997-1999 [70], five patients (1.6%) were found to have suffered acute onset dyspnoea, fever, and cough between 3 days and 3 weeks after gemcitabine exposure (and after 1-6 cycles of treatment). Chest X-rays showed diffuse interstitial/alveolar infiltrates in all five. Pulmonary function tests were undertaken in three and showed reduced TLC, CO diffusing capacity, and FEV1. All patients were treated with high dose methylprednisolone

and oxygen, with prompt symptomatic and radiological improvement. Four out of the five had received radiotherapy to the thorax during the previous 3-8 weeks; however, the distribution of infiltrates exceeded portals. Other groups have reported that high dose steroids provide effective treatment and may permit successful re-challenge. Lilly have no data on record to suggest radiotherapy after gemcitabine is problematic.

Carcinogenicity and mutagenicity: gemcitabine has induced forward mutations in murine cell lines, and is clastogenic *in vivo*. Mutagenic tests were negative using the Ames, *in vivo* sister chromatid exchange, and *in vitro* chromosomal aberration assays, and did not cause unscheduled DNA synthesis *in vitro*.

Impaired fertility: in female mice fertility was unaffected, but maternal toxicity were observed.

Foetal damage and teratogenicity: foetal damage and death have been observed in female mice at a dose 1/1300 the human dose on a mg/m^2 basis. Mammalian foetal malformations include cleft palate, incomplete ossification, fused pulmonary artery, and absence of gall bladder. Embryotoxicity is characterised by reduced foetal viability, reduced litter sizes, and developmental delays. Efficient contraception is essential. Patients should be appraised of potential hazards.

Lactation: it is not known whether gemcitabine or its metabolites are excreted in maternal milk but, given the potential risks, breast feeding is to be avoided.

Extravasation: gemcitabine is not vesicant, and there are no reports of injection site necrosis.

Gastrointestinal: nausea and vomiting are common, but are usually of mild to moderate severity. Grade 3/4 emetic toxicity is reported in less than 15% of patients. Diarrhoea is reported by 19% of patients, and stomatitis by 11%.

Hepatic: transient elevation of transaminases are seen in 70% of patients.

Renal: mild proteinuria and haematuria are common. Haemolytic-uraemic syndrome has been reported in six of 2429 patients receiving gemcitabine in clinical trials, two of whom developed it immediately after cessation of treatment. The diagnosis should be considered in the event of micro-angiopathic haemolytic anaemia, as evidenced by hyperbilirubinaemia, elevated LDH, reticulocytosis, thrombocytopoenia, and renal failure.

Hair and skin: hair loss, usually minimal, is reported by 15% of patients on single agent regimens. Rash is reported by up to 30% of patients, typically a macular or finely granular, maculopapular eruption of mild to moderate intensity involving trunk and extremities. Pruritis is reported by 13% of patients.

Oedema: is reported in 13-20% of patients, but is usually mild and only very rarely has required treatment discontinuation.

Flu-like symptoms: a mild, short-lived (2-3 days long), flu-like syndrome, comprising fever, headache, back pain, myalgia, asthenia and anorexia, afflicts up to 20% of patients, and this may be severe in 1-2%. Paracetamol may help. Cough, rhinitis, sweats or malaise are variable features.

Allergies: bronchospasm is reported in less than 2% of patients, and acute anaphylactoid reactions are rare.

Pharmacokinetics

Gemcitabine pharmacokinetics are linear and are described by a 2-compartment model. Population pharmacokinetic analyses of combined single and multiple dose studies showed that the volume of distribution of gemcitabine was significantly influenced by duration of infusion and gender. Clearance was affected by age and gender.

Differences in either clearance or volume of distribution based on patient characteristics or the duration of infusion result in changes in half-life and plasma concentrations. Table A2 shows plasma clearance and half life of gemcitabine following short infusions for typical patients by age and gender.

Table A2: **Gemcitabine clearance and half life for the "typical" female / short infusion (<70 min)**

Age	Clearance (L/hr/m^2)	Half-Life (min)
29	69.4	49
45	57.0	57
65	41.5	73
79	30.7	94

Gemcitabine half-life for short infusions ranged from 32 - 94 min, and the value for long infusions varied from 245 - 638 min, depending on age and gender, reflecting a greatly increased volume of distribution with longer infusions. The lower clearance in women and the elderly results in higher concentrations of gemcitabine for any given dose. The active metabolite, gemcitabine triphosphate, can be extracted from peripheral blood mononuclear cells. The half-life of the terminal phase for gemcitabine triphosphate from mononuclear cells ranges from 1.7 - 19.4 hrs.

Distribution: volume of distribution increases with infusion length. Volume of distribution of gemcitabine is 50 L/m^2 following infusions lasting < 70 min, indicating that gemcitabine, after short infusions, is not extensively distributed into tissues. For long infusions, the volume of distribution rose to 370 L/m^2, reflecting slow equilibration of gemcitabine within the tissue compartment. Gemcitabine plasma protein binding is negligible.

Metabolism: for pro-drug activation see *Mode of Action* above. 2'-deoxy-2', 2'-difluorouridine (dFdU) is the inactive metabolite. Maximal plasma concentrations of dFdU are achieved 30 min after discontinuation of the infusion, and the metabolite is excreted in urine without undergoing further biotransformation.

Excretion: gemcitabine disposition has been studied in five patients who received a single 1000 mg/m^2 /30 min infusion of radiolabeled drug. Within 1 week, 92- 98% of the dose was recovered, almost entirely in the urine. Gemcitabine (<10%) and the inactive uracil metabolite dFdU, accounted for 99% of the excreted dose. The metabolite does not accumulate with weekly dosing, but its elimination is dependent on renal excretion, and could accumulate with decreased renal function.

Precautions

Renal and hepatic impairment: the effects of significant renal or hepatic insufficiency on the disposition of gemcitabine have not been assessed. Laboratory evaluation of renal and hepatic function, including transaminases and serum creatinine, should be performed prior to initiation of therapy and periodically thereafter. Gemcitabine should be administered with caution in patients with evidence of significant renal or hepatic impairment.

Age: gemcitabine clearance is reduced with age, but there is no evidence that unusual dose adjustments are required.

Drug interactions: no confirmed interactions have been reported.

Radiotherapy: concurrent or intercalated gemcitabine and radical radiotherapy are to be avoided. However, there is no evidence that radiotherapy following gemcitabine exposure is problematic.

Appendix 4: Paclitaxel

Introduction

Extracts from the bark of the Pacific Yew were first identified as having therapeutic potential in 1963 in the P388 murine lymphocytic leukaemia screen. Paclitaxel (compound 17) was identified as the active constituent. Synthesis and formulation were difficult, activity was modest in pre-clinical models and the compound was accordingly afforded low priority for early clinical trials. Paclitaxel entered phase I trials in 1983. Anaphylactoid problems were successfully circumvented by adopting an extended 24 hr infusion, with steroid and antihistamine premedication. Phase II trials subsequently confirmed modest activity in platinum resistant ovarian carcinoma, and anthracycline-resistant metastatic breast cancer. Paclitaxel (Taxol®) is now manufactured by a semisynthetic process from *Taxus baccata*. The FDA has determined that semisynthetic paclitaxel is bioequivalent to that produced from Pacific Yew bark, and has approved its marketing for adjuvant therapy of node-positive early stage breast cancer, metastatic breast cancer and ovarian cancer.

Mode of action

Paclitaxel is a member of a class of cytotoxic drugs called taxanes. Taxanes promote polymerisation of tubulin and stabilise the structure of intracellular microtubules. This process inhibits the normal dynamic reorganisation of microtubules. It interferes with mitotic spindle formation during cell division and blocks the cell cycle at the metaphase/anaphase boundary. More subtly, paclitaxel impairs the dynamics of interphase microtubules, which are critical for a number of other cellular functions. Paclitaxel may also potentiate the cytotoxic effects of radiation.

Toxicity

Myelosuppression: neutropenia is the major dose limiting toxicity. It is schedule-dependent, more common with 24 hr than 3 hr infusions, and is rapidly reversible. There is no evidence of neutropenia increasing with cumulative exposure. In a phase III second lie ovarian study, infections were reported in 26% of patients treated at 175 mg/m^2 / 3hr, most commonly urinary tract and upper respiratory tract infections. Thrombocytopoenia is uncommon, and almost never severe, 7% of patients encountering platelet nadirs < 50,000. Anaemia (< 11 g/dL) is observed in 78% of patients, and is severe (< 8 g/dL) in 16%. Of all patients, 25% require transfusions. Neutrophil nadirs occur at a median of 11 days after treatment.

Cardiotoxicity: hypotension during the first 3 hrs of treatment occurs in 12% of all patients and 3% of all infusions. Bradycardia, during infusions occurs in 3% of all patients and 1% of all treatments. This risk appears independent of dose and schedule. Such episodes are usually asymptomatic, and require neither specific therapy, nor treatment discontinuation. Their incidence is unaffected by prior anthracycline therapy. Significant cardiovascular events, possibly related to paclitaxel, occur in 1% of patients and include syncope, rhythm abnormality, hypertension, and venous thrombosis. At least one syncopal episode has proved fatal. Arrhythmias include asymptomatic ventricular tachycardia, bigeminy, and complete AV block requiring pacing. Among patients with a normal ECG prior to treatment, 14% develop abnormal tracings during infusion, the most frequently reported disturbance being non-specific repolarisation abnormalities, sinus bradycardia, sinus tachycardia, and premature beats. Prior anthracycline exposure does not appear to influence the frequency of ECG abnormalities. Cases of myocardial infarction, atrial fibrillation and supraventricular tachycardia, have been reported rarely.

Pulmonary: there are rare reports of interstitial pneumonia, pulmonary fibrosis, and pulmonary embolism. There are also rare reports of radiation pneumonitis in patients receiving concurrent radiotherapy.

Mutagenicity and carcinogenicity: the carcinogenic potential of paclitaxel has not been studied. Clastogenicity has been confirmed *in vitro* and *in vivo*, but paclitaxel is not mutagenic either in the Ames test or the CHO/ HGPRT gene mutation assay. The incidence of acute myelogenous leukaemia (AML) recorded to date in both CALGB 9344 and NSABP-B28 studies is comparable with that recorded in studies exposing patients to cyclophosphamide and epirubicin without paclitaxel.

Impaired fertility: has been demonstrated in female rats at doses equivalent to those recommended for human use.

Foetal damage and teratogenicity: embryo and foetal damage has been documented in mammalian studies, with increased intrauterine mortality and foetal deaths. No teratogenic effects have been observed at doses equivalent to those recommended for use in humans, but observations were hampered by extensive foetal mortality. Effective contraception is advised of women of child-bearing potential.

Lactation: it is not known whether paclitaxel is excreted in human milk, but experiments using C^{14}-labelled paclitaxel in rats have shown higher concentration in milk than plasma. It is therefore recommended that breast feeding be avoided during paclitaxel therapy.

Thrombophlebitis: this is very rare at injection sites.

Extravasation: reactions secondary to extravasation are usually mild, consisting of erythema tenderness, and swelling at the injection site. There are no recommendations with regard to specific treatment.

Gastrointestinal: nausea or vomiting may affect up to 52% of patients but is inevitably mild to moderate in intensity. Diarrhoea affects 38%, again usually mild, as is mucositis, which typically affects about 30%.

Hair and skin: alopecia is observed in almost 90% of patients. Transient skin reactions may occur in association with hypersensitivity, these include maculopapular pruritic rashes.

Arthralgia and myalgia: experienced to some degree by 60% of all patients, but only 8% experience severe symptoms, and these are usually transient, occurring 2-3 days after treatment, and resolving after a similar period.

Peripheral neuropathy: occurs in 60% of all patients, but is severe in only 3%. Amongst patients without pre-existing neuropathy, the overall incidence is 52%, and in only 2% is it severe. The frequency of peripheral neuropathy increases with cumulative dose, and these figures may overstate the risk for this protocol, which only involves exposure to four cycles of paclitaxel. Patients with sub-clinical neuropathic conditions, such as diabetes or alcoholism, may also be at increased risk.

Acute hypersensitivity reactions: anaphalaxis and severe hypersensitivity reactions characterised by dyspnoea and hypotension requiring treatment, angioedema and generalised urticaria occur in 2-4% of patients receiving paclitaxel. Fatal reactions have occurred despite premedication, rechallenge should be avoided after severe reactions. It is now known that the frequency and severity of acute hypersensitivity reactions are unaffected by the dose or schedule of administration. The symptoms most frequently observed include dyspnoea, flushing, chest pain, and tachycardia. Guidelines for prophylaxis and treatment are included in the main section of the protocol (sections 7.4 and 7.5). Note that there is no need to withdraw patients following reactions such as rash. Indeed, such problems are often transient and do not recur with re-challenge.

Pharmacokinetics

The pharmacokinetics of paclitaxel vary considerably depending on the dosage and duration of infusion. Plasma concentrations of paclitaxel decline in a biphasic manner following injection. Increasing a 24-hr infusion from 135 mg/mL to 175 mg/mL increased C_{max} by 87% while the area under the plasma concentration-time curve (AUC) remained constant. Increasing a 3 hr infusion increased C_{max} by 68% and the AUC by 89%.

Distribution: with the 24-hr infusion of paclitaxel, the mean apparent volume of distribution in the steady state ranged from 227 to 688 L/m^2, indicating extensive extravascular distribution or tissue binding. In vitro studies show that 89- 98% of paclitaxel is bound to human serum proteins.

Metabolism: paclitaxel may undergo extensive hepatic metabolism in humans by a cytochrome P450 isoenzyme, CYP2C8. The disposition of paclitaxel in patients with renal or hepatic dysfunction has not yet been determined. Two minor metabolites are produced by CYP3A4.

Excretion: mean values for cumulative urinary recovery of unchanged drug range from 1.3–12.6% of dose, indicating extensive non-renal clearance. Studies with radiolabeled paclitaxel via 3 hr infusion have shown 71% of radioactivity to be faecally excreted, with 14% recovered from the urine. Paclitaxel represented just 5% of radioactivity recovered from faeces, whilst metabolites, primarily 6α-hydroxy paclitaxel, accounted for the majority

Precautions

Paclitaxel should not be used for patients with hypersensitivity to polyoxyethylated castor oil (Cremaphor EL) or paclitaxel. Patients should be pre-treated with corticosteroids (e.g. dexamethasone and diphenhydramine) and H2-receptor antagonists (cimetidine or ranitidine.)

There is evidence that the toxicity of paclitaxel is increased in patients with abnormally high liver enzymes and caution should be exercised when treating patients with moderate to severe hepatic impairment and dose adjustment should be considered.

Drug interactions: the metabolism of paclitaxel is catalysed by cytochrome P450 isoenzymes, and care should be exercised in co-administering known substrates or inhibitors of these enzymes. In vitro, the metabolism of paclitaxel is inhibited by ketoconazole, verapamil, diazepam, quinidine, dexamethasone, cyclosporin, etoposide, and vincristine, but the concentrations used exceeded those found *in vivo* during therapy.

Appendix 5: Guidelines for Radiotherapy

General/Timing

Irradiation should be postponed until systemic treatment is completed. Ideally, it should commence 4 weeks after the last cycle of chemotherapy commences, i.e. 3 weeks after last exposure to gemcitabine/paclitaxel. However, it should start no later than 6 weeks after the last cycle of chemotherapy.

Radiotherapy indications

Chest wall radiotherapy

Chest wall radiotherapy following mastectomy should be considered for patients who fit any one of the following criteria [67]:

- T3 and T4 tumours
- One or more axillary nodes involved
- Lymph-vascular invasion
- High grade tumours
- Close excision margin

Radiotherapy to the breast itself

This is an integral part of any breast-conserving procedure and should be performed in all cases.

Nodal radiotherapy

Radiotherapy must include the axilla if an axillary sample has been positive and surgical clearance has not been performed. In these cases, it is strongly recommended that a treatment technique is used which minimises any overlap, and that the match interface should not involve the axilla, a potential disease site.

Radiotherapy to the axilla after an axillary clearance must be avoided unless there is evidence of residual disease in the axilla.

Irradiation of internal mammary nodes should be avoided so as to minimise the radiation dose to myocardium and lung.

Extracapsular spread in patients with involved axillary nodes does not constitute an absolute indication for axillary radiotherapy after surgical clearance of the axilla, given the higher risks of lymphoedema in these circumstances, and the lack of any evidence of survival benefit. Any treatment must only be considered after careful discussion with the patient on an individual patient basis.

Another controversial area is the case for a supraclavicular field in patients with more than three axillary nodes involved, especially, perhaps, those with apical node involvement. Within this study, a particular concern relates to the potentially neurotoxic effects of paclitaxel and the theoretically increased risks of brachial plexopathy in these circumstances. However, we feel it would be inappropriate to proscribe these practices and it is recognised such decisions have to be made on a case by case basis. If fraction sizes greater than 2 Gy are used, then total dose applied to the supraclavicular field must be reduced appropriately. A suggested treatment planning protocol for this contingency may be found below. We recognise this approach is increasingly employed for an involved sentinel node, or involved node(s) at sampling, as an alternative to formal axillary clearance.

Technique

Position of the patient

The patients will be treated in the supine position. This position should be reproduced during simulation, acquirement of planning CT (if used) or contour and treatment. It is advised to assess the reproducibility by orthogonal laser beams.

Chest wall / Breast field.

Tangential fields will be used. Irradiation of large volumes of lung by the tangential fields should be avoided by keeping the central lung distance to less than 3 cm.

For patients with left-sided tumours, the irradiation of large volumes of heart must be avoided by keeping the distance from the posterior edge of the field to the anterior border of the heart to <1.5 cm. If these parameters cannot be met, then we recommend that either full CT planning or the use of a lead cardiac shield on the medial field should be used.

A simulator film or digital image must be taken on the medial field to verify the above parameters have been met. A minimum of one transverse outline, taken on the central axis of the length of the tangential fields should be taken.

Axilla and supra-clavicular field.

Where the clinician feels these are a necessity, an anterior supraclavicular field with an opposed posterior axillary field will be used. The upper border will cover the supraclavicular fossa and is about 3 cm above the head of the clavicle. It is suggested that a gantry angle (usually of about 15%) is used to angle the field away from the spine. The medial border is the ipsilateral edge of the vertebral bodies. The lower field border should be matched onto the upper border of the tangential fields. If no chest-wall fields are to be used, then the lower border of the supra-clavicular field should be at the level of the lower end of the head of the clavicle. The posterior axillary field should cover the apex of the axilla superiorly, the lower edge of anterior supraclavicular field inferiorly, and the lateral ends of the ribs medially. The use of a surgical clip is ideal to define the lower border of radiotherapy and the upper of border surgery, in the event of a level one clearance (sampling). Any shielding blocks will be indicated on a simulation film.

Supra-clavicular field.

Where the clinician feels this is a necessity, a single anterior field will be used. The infero-lateral corner should lie at the marker placed at the supra-medial limit of the axillary dissection. The upper border will cover the supraclavicular fossa and is about 3 cm above the head of the clavicle. It is suggested that a gantry angle (usually of about 15%) is used to angle the field away from the spine. The medial border is the ipsilateral edge of the vertebral bodies. The lower field border should be matched onto the upper border of the tangential fields. If no chest-wall fields are to be used then the lower border of the supra-clavicular field should be at the level of the lower end of the head of the clavical. Any shielding blocks will be indicated on a simulation film.

Dose and Fractionation

The dose distribution should be shown at least in the plane through the beam axes. The target area (PTV) in this plane should be outlined.

The tumour dose is specified at the iso-centre for the tangential fields, to the mid-plane for axillary fields and as an incident dose for the supraclavicular field.

A number of different dose/ fractionation schedules are in routine use. The following schedules are acceptable, to both the breast and nodal fields:

50 Gy / 25 daily fractions over 5 weeks

46 Gy / 23 daily fractions over 4½ weeks

45 Gy / 20 daily fractions over 4 weeks

40 Gy / 15 daily fractions over 3 weeks

For patients having had conservative surgery, a boost to the tumour bed may be given in accordance with local protocol.

Treatment verification

We recognise that NHS funding constraints mean that verification films are not part of standard practice, in contrast to much of Western Europe and North America. However, where local resources do allow, it is recommended that a weekly portal imaging film (or other recording when using on-line portal imaging systems) be obtained during the course of treatment. Portal films should be compared to the simulator film. Field adjustments should be made in case of clinically important difference. This is not a requirement of the study. It should not discourage clinicians form participating.

Alternative methods

Some centres have developed their own specific irradiation techniques for breast, chest wall, and supraclavicular treatments. Irradiation techniques and dosages differing from those described in the protocol, e.g. electron fields for chest wall irradiation, can be allowed, provided a detailed description is given.

Alternative dose schedules are allowed if these are routinely employed by any centre, but the doses must remain constant for both arms of the trial and must be described in advance. The description of any alternative techniques and/or dose/ fractionation schedules will be reviewed by the Steering Committee prior to inclusion as a trial participant.

Appendix 6: Grading System For Radiotherapy-Related Reactions

Acute skin reaction to radiotherapy [68]

Mild:	Nil reaction or mild/ moderate erythema <5% dry desquamation in field
Moderate:	Marked erythema with between 5-10% desquamation (dry or moist)
Severe:	Dry or moist desquamation in >10% of field Or treatment gap required due to skin reaction Or incomplete healing 1 month post-radiotherapy

Telangiectasia following mastectomy or wide local excision [69]

Excellent:	No telangiectasia
Good:	Minimal telangiectasia
Moderate:	Moderate telangiectasia in whole breast/ breast boost site
Fair to poor:	Severe telangiectasia in whole breast/ breast boost site

Appendix 7: ECOG Performance Status

Status description

0: Asymptomatic, fully active and able to carry out all pre-disease performance without restriction.

1: Symptomatic, fully ambulatory but restricted in physically strenuous activity and able to carry out performance of a light or sedentary nature e.g. light housework, office work

2: Symptomatic, ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours: in bed less than 50% of day.

3: Symptomatic, capable of only limited self-care, confined to bed or chair more than 50% of waking hours, but not bed-ridden.

4: Completely disabled. Cannot undertake any self-care. Totally bed-ridden

Appendix 8: Common Toxicity Criteria

Toxicity Grade	0	1	2	3	4
Cardiovascular					
Cardiac dysrhythmias	none	asymptomatic, not requiring treatment	symptomatic, but not requiring treatment	symptomatic and requiring treatment	life-threatening (e.g. arrhythmia associated with CHF, hypotension, syncope, shock)
Cardiac left ventricular function	normal	asymptomatic decline of resting ejection fraction of ≥10% but < 20% of baseline value; shortening fraction ≥24% but <30%	asymptomatic but resting ejection fraction below LLN for laboratory or decline of resting LVEF ≥ 20% of baseline value; <24% shortening fraction	CHF responsive to treatment	severe or refractory CHF or requiring intubation
Cardiac ischaemia/ infarction	none	non-specific T-wave flattening or changes	asymptomatic, ST + T-wave changes suggesting ischaemia	angina without evidence for infarction	acute myocardial infarction
Pericardial effusion/ pericarditis	none	asymptomatic effusion, not requiring treatment	pericarditis (rub, ECG changes, and/or chest pain)	with physiologic consequences	tamponade (drainage or pericardial window required)
Hypotension	none	changes, but not requiring therapy (incl. transient orthostatic hypotension)	requiring brief fluid replacement or other therapy but not hospitalisation; no physiologic consequences	requiring therapy and sustained medical attention, but resolves without persisting physiologic consequences	shock (associated with acidaemia and impairing vital organ function due to tissue hypoperfusion)
Phlebitis (superficial)	none	--	present	--	--
Thrombosis/ embolism	none	--	deep vein thrombosis, not requiring anticoagulant	deep vein thrombosis, requiring anticoagulant therapy	embolic event including pulmonary embolism
Dermatological					
Alopecia	normal	mild hair loss,	pronounced hair loss	--	--
Skin (rash/desquamation)	none	macular or papular eruption or erythema without associated symptoms	macular or papular eruption or erythema with pruritis or other associated symptoms covering <50% of body surface or localised desquamation or other lesions covering <50% of body surface area	symptomatic generalised erythroderma or macular, papular, or vesicular eruption or desquamation covering ≥50% of body surface area	generalised exfoliative dermatitis or ulcerative dermatitis
Injection site reaction	none	pain or itching or erythema	pain or swelling, with inflammation or phlebitis	ulceration or necrosis that is severe or prolonged, or requiring surgery	--

Toxicity Grade	0	1	2	3	4
Gastrointestinal					
Nausea	none	able to eat	oral intake significantly decreased	no significant intake, requiring i.v. fluids	--
Vomiting	none	1 episode in 24 hrs over pre-treatment	2-5 episodes in 24 hrs over pre-treatment	≥ 6 episodes in 24 hrs over pre-treatment, or need for i.v. fluids	requiring parenteral nutrition; or physiologic consequences requiring intensive care; haemodynamic collapse
Diarrhoea	none	increase of <4 stools/day over pre-treatment	increase of 4-6 stools /day, or nocturnal stools	increase of ≥7 stools/day, or incontinence; or need for parenteral support for dehydration	physiologic consequences requiring intensive care; or haemodynamic collapse
Constipation	none	requiring stool softener or dietary modification	requiring laxatives	obstipation requiring manual evacuation or enema	obstruction or toxic megacolon
Stomatitis	none	painless ulcers, erythema, or mild soreness in the absence of lesions	painful erythema, oedema, or ulcers but can swallow	painful erythema, oedema, or ulcers, requiring i.v. hydration	Severe ulceration requires prophylactic intubation
Oesophagitis/ dysphagia	none	mild dysphagia, but can eat regular diet	dysphagia, requiring predominantly pureed, soft, or liquid diet	dysphagia, requiring i.v. hydration	complete obstruction (cannot swallow saliva); requiring enteral or parenteral or perforation
Weight gain	< 5%	5 – <10%	10 – <20%	≥ 20%	--
Weight loss	< 5%	5 – <10%	10 – <20%	≥ 20%	--
Haematological					
WBC $\times 10^9$/L	WNL	3.0 – <LLN	≥2.0 – <3.0	≥1.0 – <2.0	< 1.0
Platelets $\times 10^9$/L	WNL	75 – <LLN	≥50 - <75	≥10 – <50	< 10
Haemoglobin g/dL g/L mmol/L	WNL WNL WNL	10.0 – <LLN 100 – <LLN 6.2 – <LLN	8.0 - <10.0 80 – <100 4.95 – <6.2	6.5 – <8.0 65 – <80 4.0 – <4.9	< 6.5 < 65 <4.0
Neutrophils/ granulocytes $\times 10^9$/L	WNL	≥1.5 – <2.0	≥1.0 – <1.5	≥0.5 – <1.0	< 0.5
Lymphocytes $\times 10^9$/L	WNL	1.0 – <LLN	≥0.5 - <1.0	<0.5	--
Infection/ fever/febrile neutropenia Catheter-related infection	none	mild, no active treatment	moderate, localized infection, requiring local or oral treatment	severe, systemic infection, requiring IV antibiotic or antifungal treatment or hospitalisation	life-threatening sepsis (e.g. septic shock)
Febrile neutropenia (fever of unknown origin without clinically or microbiologically documented infection) (ANC <1.0 $\times 10^9$/L, fever ≥38.5°C)	none	-	-	present	life-threatening sepsis (e.g. septic shock)

Toxicity Grade	0	1	2	3	4
Infection (documented clinically or microbiologically) with grade 3 or 4 neutropenia (ANC <1.0 × 10^9/L)	none	-	-	present	life-threatening sepsis (e.g. septic shock)
Infection with unknown ANC	none	-	-	present	life-threatening sepsis (e.g. septic shock)
Infection without neutropenia	none	mild, no active treatment	moderate, localised infection, requiring local or oral treatment	severe, systemic infection, requiring i.v. antibiotic or antifungal treatment, or hospitalisation	life-threatening sepsis (e.g. septic shock)
Fever in absence of neutropenia	none	38.0 – 39.0°C	39.1 – 40.0°C	> 40°C for < 24 hrs	> 40°C for > 24hrs,
Chills	none	mild, requiring symptomatic treatment (e.g. blanket) or non-narcotic medication	severe and/or prolonged, requiring narcotic medication	not responsive to narcotic medication	--
Myalgia/ arthralgia	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain: pain or analgesics severely interfering with activities of daily living	disabling
Liver					
Transaminase SGOT/AST & SGPT/ALT	WNL	>ULN-2.5 × ULN	>2.5 – 5.0 × ULN	>5.0 – 20.0 × ULN	> 20.0 × ULN
Neurological					
Neuropathy - sensory	normal	loss of deep tendon reflexes or paraesthesia (incl. tingling) but not interfering with function	objective sensory loss or paraesthesia (incl. tingling), interfering with function, but not interfering with activities of daily living	sensory loss or paraesthesia interfering with activities of daily living	permanent sensory loss that interferes with function
Mood alteration (anxiety/depression)	normal	mild mood alteration not interfering with function	moderate mood alteration interfering with function, but not interfering with activities of daily living	severe mood alteration interfering with activities of daily living	suicidal ideation or danger to self
Headache	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living transient	severe pain: pain or analgesics severely interfering with activities of daily living	disabling
Taste disturbance	normal	slightly altered	markedly altered	--	--
Fatigue (lethargy, malaise, asthenia)	none	increased fatigue over baseline, but not altering normal activities	Moderate (e.g. decrease in performance status by 1 ECOG level or 20% Karnofsky or Lansky) or causing difficulty performing some activities	severe (e.g. decrease in performance status by ≥2 ECOG levels or 40% Karnofsky or Lansky) or loss of ability to perform some activities	bedridden or disabling

Toxicity Grade	0	1	2	3	4
Ocular					
Conjunctivitis/ keratitis	none	abnormal opthalmological changes, but asymptomatic or symptomatic without visual impairment (*i.e.* pain and irritation)	symptomatic and interfering with function, but not interfering with activities of daily living	Symptomatic and interfering with activities of daily living	(keratitis) unilateral or bilateral loss of vision (blindness)
Pulmonary					
Dyspnoea	normal	--	dyspnoea on exertion	dyspnoea at normal level of activity	dyspnoea at rest or requiring ventilator support
CO diffusion capacity (DL_{CO})	≥ 90% of pre-treatment or normal value	≥75 – <90% of pre-treatment or normal value	≥50 – <75% of pre-treatment or normal value	≥25-<50% of pre-treatment or normal value	< 25% of pre-treatment or normal value
Pulmonary fibrosis	none	radiographic changes, but asymptomatic or symptoms not requiring steroids	requiring steroids or diuretics	requiring O_2	requiring assisted ventilation
Pneumonitis/ pulmonary infiltrates	none	radiographic changes but asymptomatic or symptoms not requiring steroids	Radiographic changes and requiring steroids or diuretics	Radiographic changes and requiring O_2	Radiographic changes and requiring assisted ventilation
Pleural effusion	none	asymptomatic and not requiring treatment	symptomatic, requiring diuretics	symptomatic, requiring O_2 or therapeutic thoracentesis	life-threatening (e.g. requiring intubation)
Adult respiratory distress syndrome (ARDS)	absent	--	--	--	present
Cough	absent	mild, relieved by non-prescription medication	requiring narcotic antitussive	severe cough or coughing spasms, poorly controlled or unresponsive to treatment	--
Renal/ genitourinary					
Haematuria	none	microscopic only	intermittent gross bleeding, no clots	persistent gross bleeding or clots; may require catheterisation or instrumentation, or transfusion	open surgery or necrosis or deep bladder ulceration
Sexual/ reproductive					
Libido	normal	decrease in interest	severe loss of interest	--	--
Dysmenorrhea	none	mild pain not interfering with function	moderate pain: pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain: pain or analgesics severely interfering activities of daily living	disabling
Hot flushes	none	mild or no more than 1 per day	moderate, > 1 per day	--	--

WNL: within normal limits: LLN: lower limit of normal; UNL: upper limit of normal

List of Abbreviations Used

ABPI	Association of the British Pharmaceutical Industry
AC	adriamycin + cyclophosphamide
AC-T	adriamycin + cyclophosphamide followed by Taxol
ALT	alanine transaminase
AML	acute myeloid leukaemia
ANC	absolute neutrophil count
ASCO	American Society of Clinical Oncology
AST	aspartate transaminase
AUC	area under the plasma concentration curve
AV	atrioventricular
b.d.	twice a day
BASO	British Association of Surgical Oncologists
BCIRG	Breast Cancer international Research Group
C.I.	confidence interval
CALGB	Cancer and Leukemia Group B
CHF	congestive heart failure
CMF	cyclophosphamide + methotrexate + 5-fluorouracil
CO	carbon monoxide
CR	complete response
CT	computerised tomography
CTC	Common Toxicity Criteria
CXR	chest X-ray
DFS	disease-free survival
DSMC	Data and Safety Monitoring Committee
EBCTCG	Early Breast Cancer Trialists' Collaborative Group
EC	epirubicin + cyclophosphamide
ECG	electrocardiogram
EORTC	European Organisation for Research and Treatment of Cancer
ER	oestrogen receptor
FBC	full blood count
FDA	Food and Drug Administration (USA)
FEC	5-fluorouracil + epirubicin + cyclophosphamide
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
(rh)G-CSF	(recombinant human) granulocyte colony stimulating factor
GFR	glomerular filtration rate
Gy	Gray
Hb	haemoglobin
HER2	human epidermal growth factor receptor-2
HR	hazard ratio
HRT	hormone replacement therapy
ICH	International Conference on Harmonisation (GCP)
ICPA	International Co-operative Project Assurance
i.m.	intramuscular
i.v.	intravenous
IU	international unit
K_{CO}	transfer coefficient for carbon monoxide
LDH	lactate dehydrogenase
LLN	lower limit of normal
LREC	Local Research Ethics Committee
LVEF	left ventricular ejection fraction
MREC	Multi-centre Research Ethics Committee
MTD	maximum tolerated dose
MUGA	multi-gated radionuclide angiography
NHS	National Health Service
NICE	National Institute for Clinical Excellence
NIH	National Institute of Health (USA)
ns	not significant
NSABP	National Surgical Adjuvant Breast and Bowel Project
NSCLC	Non-small cell lung cancer
OPRR	Office for the Protection of Research Risks
OR	overall response
OS	overall survival
p.o.	by mouth
PgR	progesterone receptor
PR	partial response
PTV	planned target volume
q	every
R&D	research and development
SAE	serious adverse event
SD	standard deviation
SGOT	serum glutamic oxalacetic transaminase
SGPT	serum glutamic pyruvic transaminase
t.d.s.	three times a day
T_{LCO}	transfer factor for carbon monoxide
TLC	total lung capacity
TTP	time to progression
ULN	upper limit of normal
WBC	white blood cell count
WMA	World Medical Association
WNL	within normal limits

R A N D O M I S A T I O N

Between 9 am and 5 pm, Monday to Friday

tAnGo Study Office
Co-ordinating trials unit:

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tAnGo is supported by Cancer Research UK and was awarded a high alpha rating by the Clinical Trials Committee following independent peer review.

This trial has the support of the NCRI Breast Cancer Clinical Studies Group which considers the goal to be valid and important, and the design to be appropriate. The Group will strongly encourage recruitment to the study through the NCRN.