Gemcitabine added to paclitaxel-containing, epirubicin / cyclophosphamide-based, adjuvant chemotherapy for women with early stage breast cancer (*tAnGo*): an open-label, randomised, phase 3 trial

^Helena M Earl[1,2,3], ^Louise Hiller[4], Helen C Howard[5], Janet A Dunn[4], Jennie Young[6], Sarah J Bowden[6], Michelle McDermaid[7], Anna Waterhouse[8], Gregory Wilson[9], Rajiv Agrawal[10], Susan O'Reilly[11], Angela Bowman[12], Diana Ritchie[13], Andrew Goodman[14], Tamas Hickish[15], Karen McAdam[16,17], David Cameron[12], David Dodwell[18], Daniel Rea[6], Carlos Caldas[1,2,3,19], Elena Provenzano[2,3], Jean Abraham[1,2,3], Peter Canney[20], John Crown[21], John Kennedy[22], Robert Coleman[23], Robert C Leonard[24], James Carmichael[25], *Andrew M Wardley[26], *Christopher J Poole[27], on behalf of the *tAnGo* trial Collaborators.

1. University of Cambridge, Department of Oncology, Box 193 (R4), Addenbrooke's Hospital, Hills Road, Cambridge, CB2 0QQ. UK
2. NIHR Cambridge Biomedical Research Centre, Box 277, Hills Road, Cambridge, CB2 0QQ. UK
3. Cambridge Breast Cancer Research Unit, Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ. UK
4. Warwick Clinical Trials Unit, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL. UK
5. Leeds Institute for Clinical Trials Research, University of Leeds, Leeds, LS2 9JT. UK
6. University of Birmingham, Cancer Research UK Clinical Trials Unit (CRCTU), Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, B15 2TT. UK
7. NHS National Services Scotland, Scottish Clinical Trials Research Unit, Gyle Square, South Gyle Crescent, Edinburgh, EH12 9EB. UK

8. University of Leeds, Leeds Institute of Clinical Trials Research, University of Leeds, Leeds, LS2 9JT. UK

9. The Christie NHS Foundation Trust, Medical Oncology, 550 Wilmslow Road, Manchester, M20 4BX, UK

10. Shrewsbury & Telford NHS Hospitals Trust, Department of Oncology, Mytton Oak Road, Shrewsbury, SY3 8XQ. UK

11. Clatterbridge Cancer Centre, Department of Oncology, Clatterbridge Road, Wirral, Merseyside, CH63 4JY. UK

12. University of Edinburgh, Cancer Research Centre, IGMM, Western General Hospital, Crewe Road South, Edinburgh, EH4 2XR. UK

13. Beatson West of Scotland Cancer Centre, Department of Oncology, 1052 Great Western Road, Glasgow, G12 0YN. UK

14. Royal Devon and Exeter NHS Foundation Trust, Exeter Oncology Centre, Royal Devon and Exeter NHS Foundation Trust, Barrack Road, Exeter, EX2 5DW. UK

15. Poole Hospital NHS Foundation Trust/Bournemouth University, Department of Oncology, Poole Hospital , Longfleet Road, Poole, Dorset, BH15 2JB. UK

16. Cambridge University Hospitals NHS Foundation Trust, Hills Road, Cambridge, CB2 0QQ. UK

17. Peterborough and Stamford Hospitals NHS Foundation Trust, Peterborough City Hospital, Edith Cavell Campus, Bretton Gate, Peterborough, PE3 9GZ. UK

18. Leeds Teaching Hospital NHS Trust, Institute of Oncology, Level 4 - Bexley Wing, St James Hospital, Leeds, LS9 7TF. UK

19. University of Cambridge, Cancer Research UK Cambridge Institute, Li Ka Shing Centre, Robinson Way, Cambridge, CB2 0RE. UK.

20. CACTUS CRC Clinical Trials Unit, Beatson Oncology Centre, 1053 Great Western Rd, Glasgow G12 0YN. UK

21. St Vincent's University Hospital, Department of Medical Oncology, Elm park, Dublin 4, Ireland

22. Cancer Trials Ireland (formerly ICORG), 60 Fitzwilliam Sq, Dublin 2. Ireland

23. University of Sheffield, Weston park Hospital, Sheffield, S10 2SN. UK

24. Imperial College, Department of Surgery and Cancer, Charing Cross Hospital, Fulham Palace Road, London W6 8RF

25. Celgene, Research and Development, 10300 Campus Point Drive, San Diego, CA 92121, USA
26. The NIHR CRUK Christie Clinical Research Facility, 550 Wilmslow Road, Manchester M20 4BX. UK
27. Arden Cancer Centre, University Hospital Coventry and Warwickshire, CV2 2DX. UK

Short title: *tAnGo*: An adjuvant breast cancer trial of sequential EC-GT

^Joint first and corresponding authors

Professor Helena Earl, Professor of Clinical Cancer Medicine, University of Cambridge Department of Oncology, Addenbrooke's Hospital, Box 193 (R4), Hills Road, Cambridge CB2 0QQ, UK. hme22@cam.ac.uk 01223 336800

Louise Hiller, Principal Research Fellow, Warwick Clinical Trials Unit, University of Warwick, Gibbet Hill Road, Coventry, CV4 7AL. UK. l.hiller@warwick.ac.uk 02476 150179

*Joint senior authors

ABSTRACT

Background: *tAnGo,* an international phase III trial, was designed to evaluate the potential role of gemcitabine when added to anthracycline and taxane-containing adjuvant chemotherapy for early breast cancer (EBC). At the time the study was developed gemcitabine had shown significant activity in metastatic breast cancer, and there was evidence of a favourable interaction with paclitaxel.

Methods: *tAnGo* was a superiority trial and aimed to detect 5% differences in 5-year DFS rates with EC-GT (4 cycles of epirubicin 90mg/m^2 IV and cyclophosphamide 600mg/m^2 IV day1 every (q) 3 weeks, followed by 4 cycles of paclitaxel 175mg/m^2 /3hour infusion day1 and gemcitabine 1250mg/m^2 IV days1 and 8 q3 weeks) when compared with EC-T, in women with invasive EBC, with a definite indication for adjuvant chemotherapy. Disease-free survival (DFS) was the primary endpoint and overall survival (OS) a secondary endpoint. Stratification was by country, age, radiotherapy intent, nodal status, oestrogen (ER) and HER2 status. This trial is registered with EudraCT (2004-002927-41), ISRCTN (51146252), and ClinicalTrials.gov (NCT00039546).

Results: Between August 2001 and November 2004, 3152 patients were randomised from the UK and Ireland, by 127 centres; 1576 to EC-GT, and 1576 to EC-T. Patient characteristics were balanced across treatment groups: 77% node positive, 55% $\leq$50 years old, 62% of tumours grade 3, 63% >2cm, 44% ER negative, 50% PgR negative, 13% HER2 positive. This protocol-specified final analysis has a median follow-up of 10 years (IQR 10-10 years) and recorded 1087 DFS events and 914 deaths. No statistically significant difference between treatments was observed in DFS (adjusted HR=0·97 (95%CI 0·86-1·10) p=0·64) or OS (adjusted HR=1·02 (95%CI 0·89-1·16) p=0·81). No benefit for EC-GT was found in any of the protocol-determined subgroups. Toxicity, dose intensity and the detailed safety sub-study showed both regimens to be safe, deliverable and tolerable.

Conclusions: The addition of gemcitabine to anthracycline and taxane-based adjuvant chemotherapy at this dose and schedule confers no therapeutic advantage in terms of DFS and OS in early breast cancer.

Key Words: *tAnGo*, early breast cancer, adjuvant chemotherapy, EC-T, gemcitabine.

INTRODUCTION

Despite the therapeutic advances of the last three decades, the development of more effective adjuvant therapy remains a priority for improving the treatment of women with early breast cancer The modest impact of traditional adjuvant polychemotherapy (mainly cyclophosphamide, methotrexate, 5-fluoruracil [CMF]) had been confirmed as a 24% global reduction in the risk of relapse or death (hazard ratio (HR)=0·76) and a 15% reduction in the risk of death (HR=0·85) in the meta-analysis by the Early Breast Cancer Trialists' Collaborative Group[1]. The incorporation of anthracyclines provided additional benefits, with an estimated HR for relapse or death of 0·88 compared with CMF[1], and a HR $\leq$ 0·7 in individual trials with higher dose epirubicin-based adjuvant regimens[2,3,4]. Following the routine inclusion of anthracyclines into standard adjuvant treatment, both the CALGB 9344[5] and NSABP B28[6] trials showed that the sequential addition of four cycles of paclitaxel to standard therapy with four cycles of doxorubicin and cyclophosphamide further reduced the risk of recurrence. CALGB 9344 reported that the risk of relapse or death was reduced by 17% (HR=0·83 (95%CI 0·73-0·94), p=0·0013) and the risk of death by 18% (HR=0·82, (0·71-0·95), p=0·0061)[5] The NSABP B28 trial confirmed a HR=0·83 (0·73-0·95), p=0·008) for relapse or death and a non-significant improvement in overall survival. A meta-analysis of polychemotherapy conducted by the EBCTCG confirmed the benefits of the addition of taxanes to anthracycline-based polychemotherapy regimens[7].

Based on pre-clinical evidence of a potentially favourable interaction between paclitaxel and gemcitabine, and the favourable results of a pivotal randomised phase III trial comparing paclitaxel and gemcitabine in combination against single agent paclitaxel in patients who had anthracycline pre-treated metastatic disease, it seemed plausible that the addition of gemcitabine to paclitaxel in the anthracycline / cyclophosphamide (AC-T) regimen might

further improve DFS and OS in early stage disease. The *tAnGo* trial was designed to evaluate the addition of gemcitabine to a sequential epirubicin and cyclophosphamide followed by paclitaxel regimen. The only other adjuvant trial in breast cancer testing the addition of gemcitabine to standard chemotherapy was NSABP-B38[8], a three arm trial with gemcitabine added to doxorubicin /cyclophosphamide and paclitaxel (AC-TG).

The *tAnGo* trial was one of the first breast cancer trials to have a 'companion' neoadjuvant study (Neo-*tAnGo*) which reported in 2014[9]. Both the endpoint of pathological complete response (pCR) from the Neo-*tAnGo* trial, which directly tests the chemo-sensitivity of the combination on the primary tumour, and the long term outcomes of DFS and OS from the *tAnGo* trial reported in this manuscript, are now available.

A preliminary safety analysis without outcome data on the first 135 patients reported in 2008, demonstrated that both regimens were well tolerated, with only temporary changes in pulmonary function and transaminitis[10]. The preliminary results of *tAnGo* were presented as a conference abstract in 2008 at a median of 3 years follow-up[11], and showed no benefit from the addition of gemcitabine (DFS HR=1·0 (95%CI 0·8-1·2), p=0·96, OS HR=1·1 (95%CI 0·9-1·4), p=0·35). The present manuscript provides the only definitive and final analysis of the trial, All *tAnGo* patients were randomised more than 10 years ago, and we report here the long-term results for DFS and OS at a median follow-up of 10 years (IQR 10-10 years) alongside the 5- and 10-year detailed safety study findings.

METHODS

Study design and participants

The *tAnGo* phase III randomised trial was designed to test the hypothesis that the addition of gemcitabine to the second phase of a standard regimen of epirubicin and cyclophosphamide followed by paclitaxel (EC-GT) improves disease-free survival (DFS) in comparison to epirubicin and cyclophosphamide followed by paclitaxel alone (EC-T) in women with early stage breast cancer with a definite indication for adjuvant chemotherapy.

We enrolled women aged 18 years or older with completely excised invasive early breast cancer of any nodal or hormone receptor status. Patients were enrolled where according to risk, a definite indication for adjuvant chemotherapy existed. This included ER negative (defined as Allred score 0-2); ER weakly positive (Allred score 3-5); and grade 3 tumours, and in these categories this was regardless of nodal status. Patients with ER strongly positive (Allred Score 6-8) or grade I or II tumours were usually included only if positive axillary lymph nodes were present. Women were enrolled at 127 sites in the UK and 2 in Ireland. Other eligibility criteria included adequate bone marrow, hepatic, and renal function, adequate (0-1) ECOG performance status, no previous exposure to chemotherapy or radiotherapy, no previous or concomitant cancer, the ability to commence chemotherapy within 8 weeks of surgery, and written informed consent.

At the start of the trial, eligibility criteria stated that tumours must be either ER-negative (defined as Allred score 0-2) or weakly positive (Allred score 3-5) and, in the case of weakly ER-positive tumours, these must be either PgR-negative or weakly positive (Allred score 3-5). In 2003, after 550 patients had been recruited, the protocol was amended and these initial criteria were relaxed to include patients with any hormone receptor status, given the

evidence that was accruing during the trial recruitment phase, for taxane activity irrespective of hormone receptor status. Full eligibility can be found in the trial protocol.

The *tAnGo* trial was an investigator designed and led trial, approved by the MHRA on 06-Sep-2000, the West Midlands Multi-Centre Research Ethics Committee on 11-Dec-2000 and by all Local Research Ethics Committees and Research and Development Departments at participating hospitals. The trial was centrally coordinated by the Cancer Research UK Clinical Trials Unit, University of Birmingham with regional coordination being provided by the Clinical Trials Research Unit, University of Leeds, the ISD Cancer Clinical Trials Team, Partner in CaCTUS in Edinburgh and Cancer Trials Ireland (formerly the All Ireland Cooperative Oncology Research Group - ICORG), Dublin, Ireland. Statistical support was provided by Warwick Clinical Trials Unit, University of Warwick.

Randomisation and masking

The open-label *tAnGo* trial used a central computerised deterministic minimisation procedure to randomise patients (1:1) between EC-GT and EC-T treatment regimens (Figure 1). Treatment allocation was made by telephone to one of the three regional trials offices (Birmingham, Leeds or Edinburgh). Stratification was by country (England, Scotland, Wales, Republic of Ireland, Northern Ireland), age (<=50, >50 years old), radiotherapy intent (planned, not planned), nodal status (negative, 1-3 nodes positive, 4+ nodes positive), ER status (negative, weakly positive, strongly positive) and HER2 status (3+, other [0, 1+, 2+], unknown).

Procedures

The primary endpoint was disease-free survival and secondary endpoints were overall survival, toxicity, delivered dose-intensity, tolerability and serious adverse events. We assessed adverse events for each chemotherapy cycle according to Common Terminology Criteria for Adverse Events (CTCAE) v2.0 grade.

If neutropenic fever or sepsis occurred after a cycle of chemotherapy, the next cycle was delayed until the absolute neutrophil count was at least $1{\cdot}0 \times 10^9$ cells per L. Following a delay, either dose reduction of all drugs to 80%, or GCSF support with 100% dose were allowed, and all remaining cycles of the same four-cycle block were given at those doses. For persistent thrombocytopenia, the next cycle was delayed until patients had at least 100 $\times\ 10^9$ platelets per L and was reduced to 80%, maintaining this dose reduction for subsequent cycles. Primary prophylaxis with GCSF was not provided with either epirubicin and cyclophosphamide or paclitaxel (with or without gemcitabine). Once started, prophylactic GCSF was usually continued into the second phase of chemotherapy at the discretion of the responsible physician. Day 8 FBC values had no impact on treatment decisions.

If grade 2 neuropathy occurred during treatment with paclitaxel, remaining doses were reduced to 135 mg/m² (gemcitabine was unchanged). If grade 3 neuropathy occurred, either gemcitabine continued alone or trial chemotherapy was discontinued.

Gemcitabine was dose-reduced to 80% in the event of grade 3 hepatic toxicity (transaminitis; aspartate aminotransferase or alanine aminotransferase ≥5–20 × upper limit of normal [ULN]) on day of treatment at clinician's discretion, because transaminitis is not known to

affect gemcitabine clearance. We were unable to substantiate earlier concerns about gemcitabine's potential for clinically significant hepatic impairment. In the event of gemcitabine-related pulmonary toxicity of CTCAE grade 2 or worse, the patient was discontinued from study therapy.

Cardiac toxicity was not anticipated at the cumulative epirubicin dose of 360 mg/m² but if congestive cardiac failure developed, patients were investigated and treated as appropriate, epirubicin was discontinued, and other chemotherapy was given at the discretion of the treating clinician.

For allergic reactions to paclitaxel, the infusion was stopped if mild symptoms of skin rash, flushing, and localised pruritus occurred (Grade 1 and 2). Intravenous steroids and antihistamines were given and immediate slow re-challenge of chemotherapy was used on recovery. Paclitaxel infusion was stopped if moderate symptoms of generalised pruritus or rash, mild dyspnoea, or mild hypotension occurred and intravenous steroids and antihistamines were given (Grade 3). 48 h of steroids were then advised before cautious paclitaxel re-challenge. If severe symptoms occurred, including bronchospasm, generalised urticaria, angio-oedema, hypotension (systolic blood pressure <100 mm Hg), or life-threatening anaphylaxis (Grade 4), paclitaxel infusion was stopped and treatment was given with intravenous steroids, and intravenous antihistamines and if necessary intramuscular epinephrine 1 mL 1:1000; re-challenge was not recommended.

Radiotherapy was given according to local protocols, with radiotherapy intent employed as a stratification factor (planned at randomisation, not planned at randomisation). Any endocrine treatment was to be stopped prior to commencing chemotherapy, and

recommended as appropriate afterwards according to local protocols. The *tAnGo* trial was completed before adjuvant trastuzumab was used routinely and therefore the protocol did not include guidelines for adjuvant trastuzumab in HER2 positive patients. Patients could be recruited into the HERA Trial[12]. Clinical surveillance was continued for 10 years at the clinical centres.

Pathology, Immunohistochemistry (IHC) and Fluorescent in situ Hybridisation (FISH)

To investigate standard prognostic markers and treatment interactions, routine pathology tissue blocks from surgery were retrieved for 2462 of the 3141 eligible patients (78%) and were reviewed centrally in Cambridge (EP) for breast cancer morphology, tumour grade, histotype and scoring of ER, PR and HER2 on immunohistochemistry (IHC). Tissue microarrays (TMAs) were constructed with a single 0·6mm core from a representative part of the tumour (172 sample arrayed in each TMA block) and sections stained for ER, PR and HER2 by IHC, with additional FISH for those in the HER2, 2+ category. When central testing was not available (22%), results from the local report were included for the biomarker analysis.

Detailed Safety Sub-study

Cardiac, pulmonary and hepatic function were initially monitored at four time points (randomisation, mid-chemotherapy, post-chemotherapy and 6-months post-chemotherapy) and showed the treatment regimens as equally well tolerated, only causing mild to moderate reduction in pulmonary function, which recovered completely by 6 months, and gemcitabine causing increased levels of liver transaminases but no adverse clinical events[10]. For the evaluation of long-term toxicity, these assessments were also undertaken at 5 and 10 years post-treatment.

Statistical Analysis

The original sample size calculations for *tAnGo* assumed a 5-year DFS of 45-55% from patients randomised onto the control arm of the trial, given that all patients were to be ER negative. Using this, 3000 patients were deemed necessary to detect (with 5% two-sided significance,) differences in survival rates in excess of 5% with 80% power. This would also allow detection of differences in excess of 7% with 85% power and in excess of 10% with 99% power.

When the eligibility criteria for *tAnGo* were changed in September 2003, to include lower risk patients who were ER positive and PgR either positive or unknown, 550 patients had already been randomized. The effect on the expected 5-year DFS of the control arm of the trial was assessed, along with the most up-to-date 5-year DFS estimates for early stage breast cancer based on the recent CALGB 9344 and NEAT results. Following discussions with the trial Data and Safety Monitoring Committee (DSMC), it was determined that the 5-year DFS estimate for control arm patients was approximately 70% and that the power of the study to detect in excess of 5% differences with 5% two-sided significance had in fact increased from 80% to 85%. *tAnGo* thus continued to aim for the 3000 patient target.

Disease-Free (DFS) and Overall survival (OS)

The primary outcome measure of DFS was calculated from date of randomisation to date of first relapse (loco-regional or distant, not including DCIS); to date of death in women dying without relapse; or to date of censor in women alive and relapse-free. The secondary outcome of overall survival was calculated from date of randomisation to date of death, or date of censor if alive. Survival curves were constructed using Kaplan-Meier methodology. Log-rank tests assessed differences in patient and tumour characteristics, and

treatment. Cox-proportional hazards modelling investigated and adjusted for prognostic factors. Hazard ratios of treatment effects on the risk of relapse and death were calculated for prognostic subgroups and displayed as forest plots. Secondary outcome measures were adverse effects and dose intensity. A sensitivity analysis, of breast cancer-specific survival censoring deaths not due to breast cancer to the patient's date of death, was also planned.

Adverse Events

The maximum Common Toxicity Criteria (CTC) grades for a list of common toxicities reported for all patients during their chemotherapy was examined.

Dose Intensity

The methods for dose intensity calculations have previously been described[13]. We compared course delivered dose intensities (CDDI) across treatment groups with Wilcoxon rank sum tests and chi-squared tests with continuity corrections.

Pre-planned, protocol-stated primary endpoint analyses

Using Pocock's method of assigning equal weighting to the alpha spend, with significance determined by p=0·022[14], three event driven, primary endpoint analyses were planned: the first at 18 months minimum follow-up (min FU, 280 events expected) allowing detection of differences in excess of 10% with 95% power; the second at 30 months min FU (550 events expected) allowing detection of differences in excess of 7% with 95% power; the third at 60 months min FU (920 events expected) allowing detection of differences in excess of 5% with 90% power.

In June 2006, the *tAnGo* DSMC scrutinised the first pre-planned primary endpoint analysis and considered it too early to release the results. In November 2007, the second pre-planned analysis showed the conditional power[15] was 7%, below the 10-15% level pre-set by the DSMC thus making it very likely that the final results would be consistent with the current results. The DSMC therefore recommended the trial data be released and they were presented at ASCO 2008[11]. Results of the third and final pre-planned primary endpoint analysis (minimum 60 months FU, 920 events expected) are presented here.

Statistical analysis was undertaken by Warwick Clinical Trials Unit, using SAS statistical software (version 9·4). All reported p-values are two-sided. All patients who were protocol violators were analysed within their randomised groups thus allowing analysis to be undertaken on an intention-to-treat basis. *tAnGo* is registered with ISRCTN (51146252), EudraCT (2004-002927-41), and ClinicalTrials.gov (NCT00039546).

Role of the funding source

The trial was endorsed by Cancer Research UK and supported by CRUK Clinical Trials Unit core infrastructure and a grant from Breast Cancer Relief. In addition unrestricted educational grants were provided by Eli Lilly and Company Limited, Pfizer Limited and Bristol-Myers Squibb Pharmaceuticals Limited. Gemcitabine (Gemzar[TM]) and Paclitaxel (Taxol[TM]) were provided free of charge by Eli Lilly and Company Limited and Bristol-Myers Squibb Pharmaceuticals Limited respectively. Neither the sponsors of the study nor the pharmaceutical companies had any role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors (HE and LH) had full access to all of the data and (with CJP) had final responsibility for the decision to submit for publication.

RESULTS

Patients

tAnGo recruited 3152 patients from 175 clinicians at 127 centres in the UK and Ireland between August 2001 and November 2004. Eleven patients were ineligible for the trial (6 EC-GT, 5 EC-T), principally for pre-existing metastases found after randomisation, leaving 3141 eligible patients for analysis (Figure 1). Patient characteristics, type of operation, timing of surgery (Table 1) and tumour characteristics (Supplementary Table 1) for the 3141 eligible patients (1570 EC-GT, 1571 EC-T) appear balanced between the two treatment groups.

Treatment Compliance

Protocol violations in treatment allocation were noted by the trial management committee in 19 patients (7 EC-GT, 12 EC-T) (Figure 1). All analyses included these patients in their original randomised treatment group, according to the intention-to-treat principle.

Use of other first-line treatments

Of the 287 pts known to be HER2 positive, 118 (41%) were reported, at some point on their FU forms, to have received trastuzumab (54 EC-GT pts (40% of 135), 64 EC-T pts (42% of 152)), of which 60/287 (21%) received trastuzumab as adjuvant treatment and 58/287 (20%) for relapsed disease. At baseline, radiotherapy was planned to be administered in 90% of patients (2823/3141; 90% (1412/1570) of EC-GT patients, 90% (1141/1571) of EC-T patients). Radiotherapy administration is unknown in 15 patients. In the remaining 3126 patients, 2754 (88%) are recorded as having received radiotherapy treatment. Rates are equal across treatment arms (1378 (88%) EC-GT patients and 1376 (88%) EC-T patients receiving radiotherapy, p=0.99).

Disease-Free and Overall Survival

At the data lock on 29th September 2016, 914 patients had died (29% of the 3141 eligible patients; 459/1570 (29%) EC-GT patients; 455/1571 (29%) EC-T patients: Figure 1). Breast cancer was listed most frequently as the main cause of death (792/914 (87%), Table 2), but for the 122 patients for whom breast cancer was not listed as the main cause of death, 40/122 (33%) had already had a breast cancer relapse. Therefore 82 patients (9% of 914) died without evidence of recurrent breast cancer. The median follow-up for the 2227 pts recorded as being alive was 10 years (IQR 10-10 years), with 96% having more than 8 years follow-up. Loco-regional and/or distant relapse was recorded in 995 (32%) women (31% EC-GT, 32% EC-T), with distant metastases being predominantly in the bone, liver and/or lung. There were 1087 (35% of 3141) events in the analysis of DFS. 213 patients (7%) had second primaries recorded.

There was no significant difference between treatment groups in terms of DFS (log-rank p=0·63, adjusted hazard ratio (HR) 0·97 (95%CI 0·86-1·10), adjusted p=0·64, Figure 2a). DFS rates at 2, 5 and 10-years were similar for EC-GT and EC-T patients (2-year 88% vs 87%; 5-year 75% vs 74%; 10-year 65% vs 65% respectively). There were also no significant differences between treatment groups observed in OS (log-rank p=0·85, adjusted HR 1·02 (95%CI 0·89-1·16), adjusted p=0·81, Figure 2b). OS rates at 2, 5 and 10-years were similar for EC-GT and EC-T patients (2-year 93% vs 94%; 5-year 82% vs 82%; 10-year 70% vs 71% respectively). A sensitivity analysis of breast cancer-specific survival across treatment groups, analysing the 792 deaths due to breast cancer showed similar results (389 EC-GT, 403 EC-T), log-rank p=0·66, adjusted HR 0·97 (95%CI 0·85-1·12), adjusted p=0·71).

Univariate analysis showed that the following were significantly associated with worse DFS: higher nodal burden, negative ER/PR status, no radiotherapy planning, larger tumour size higher tumour grade, the presence of vascular/lymphatic invasion, use of breast-conserving surgery, (all p<0·0001), poorer ECOG performance status (p=0·004), positive HER2 status (p=0·0026), and triple negatives phenotype (p=0·01) (Table 3). Similar results were found for OS (Supplementary Table 2).

Plots of hazards over time for DFS and OS highlighted the similarity of the two randomised treatment arms (Figure 3a and 3b). However, for the HER2 negative patients, we demonstrated the expected significant differences between the ER negative sub-group (TNBC) and the ER positive subgroup in hazards over time; TNBC patients showed increased hazards for relapse and death in the early years, with a plateau of risk between 5 and 10 years, whilst ER positive patients showed persisting, albeit lower risks, for relapse and death at 10 years. In the smaller HER2 positive group ER negative and ER positive sub-groups showed a similar pattern of changes with increased risk of relapse in the early years for both, although higher hazards for ER negative patients. Of note, only 21% of HER2 positive patients received adjuvant trastuzumab.

Interaction of Treatment Effect with Prognostic Factors

Forest plots confirmed the lack of treatment effect on DFS in all subgroups of patients, specifically by age, ER, PR, nodal and HER2 status, performance status, surgery, tumour size, grade, and presence or absence of vascular or lymphatic invasion, triple negative status and ER/PGR negative status (Figure 4A-4C). Additionally no significant interactions with treatment effect were noted for these variables. Similar results were obtained for overall survival (Figure 4D-4F). A non-preplanned analysis was also carried out by four subgroups

defined in the Neo-*tAnGo* manuscript[9] using Grade 3 patients only and splitting by ER combined with HER2 status. These four subgroups showed significant heterogeneity in DFS (p=0·02) and borderline heterogeneity in OS (p=0·06), with a numerical trend for benefit from gemcitabine in the ER-/HER2+/G3 and ER-/HER2-/G3 subgroups (Figure 4C and 4F).

Adverse Effects of Chemotherapy

Information regarding adverse effects was available from 3132 patients with full sets of treatment forms returned (99·7% of the 3141 eligible patients). Frequencies of patients reporting grades 3 and 4 toxicities are as expected (Table 4). More EC-GT than EC-T patients are seen to report grade 3 neutropenia (21% vs 14%), grades 2 and 3 fatigue (55% vs 50% grade 2, 13% vs 9% grade 3), grade 3 infection (12% vs 8%) and grades 2 and 3 vomiting (25% vs 21% grade 2, 9% vs 6% grade 3). Grade 2 anaemia is also reported by more EC-GT patients (20% vs 8%).

In total, 1158 serious adverse events (SAEs) were reported (650 during EC, 136 during T and 372 during GT cycles), involving 816 patients (474 EC-GT patients, 342 EC-T patients). 1121 (97%) of these were evaluated as serious adverse reactions (SARs), involving 794 patients. There were 31 suspected unexpected serious adverse reactions (SUSARs) recorded (20 by EC-GT patients, 11 by EC-T patients).

Detailed Safety Sub-study – Long-term toxicity assessments

There were 135 patients (69 EC-GT, 66 EC-T) included in the detailed safety sub-study. The assessment completion rate at 5-years post treatment was 75% (73 of the 97 patients alive; 72% EC-GT (34 of the 47 alive) and 78% EC-T (39 of the 50 alive)). At 10-years, assessment completion rates were 74% (63 of the 85 patients alive; 78% EC-GT (31 of the 40 alive) and

71% EC-T (32 of the 45 alive)). In total, 49 patients completed all 6 assessments (58% of the 85 alive at 10-years; 25 (63%) EC-GT, 24 (53%) EC-T). There were no long-term safety signals of significant concern (Supplementary Table 3).

Early Deaths

19 patients (0·5% of the 3141 eligible patients) died within 3 months of completing their last chemotherapy cycle (10 EC-GT, 9 EC-T). 14 of these patients (7 EC-GT, 7 EC-T) died with metastatic breast cancer more than 30 days after day 1 of their last chemotherapy cycle and this indicates that they were likely to have had metastatic disease at the time of randomisation. In addition, one EC-T patient died of a second primary lung cancer and one EC-GT patient by suicide. Chemotherapy possibly contributed to 3 deaths: one (EC-GT) with breast cancer on day 18, cycle 2 from venous thrombo-embolism (VTE); one (EC-T) died from VTE 41 days after day 1, cycle 3; and one (EC-GT) from ischaemic heart disease 28 days after day 1 of cycle 2.

Dose Intensity

Complete information for dose intensity calculations was available on 3137 of the 3141 eligible patients (99·9%). EC-GT patients received moderately lower course-delivered dose intensity (CDDI) than EC-T patients (median (IQR) 96% (88-99) vs 98% (93-100), p<0·0001). Additionally, fewer EC-GT patients received CDDI ≥85% (80% vs 89%, for EC-T patients, p<0·0001).

DISCUSSION

The *tAnGo* trial showed no benefit in either DFS or OS from the addition of gemcitabine to standard paclitaxel-containing, epirubicin/cyclophosphamide-based adjuvant chemotherapy in early breast cancer. We have the benefit of data from the tenth annual follow-up on 2121 patients (95% of the 2227 known to be alive) and therefore the results for all stratified risk groups are robust and are not unduly biased by length of follow-up[16]. The *tAnGo* trial was an 'all-comers' trial carried out before the standard use of adjuvant trastuzumab for HER2 positive disease. At the start of the trial, adjuvant chemotherapy in the UK included paclitaxel for high-risk disease in very few centres. With the intention of ensuring that the results of the trial would be relevant in the future, we included paclitaxel in the standard arm, and at the start of the trial recruited only ER-negative and ER weakly positive patients, representing the highest risk population. In the second part of the trial the entry criteria were expanded to include moderate risk patients, because of emerging evidence at that point for more routine use of adjuvant taxanes[5,6].

It is difficult to explain why the overall results were negative for adjuvant gemcitabine when the drug had been so promising in the metastatic setting[17], particularly given the ongoing positive results in metastatic TNBC[18]. Gemcitabine has recently been included in an international trial in metastatic TNBC with carboplatin and *nab*-paclitaxel [19,20][HE1]. The first results of the Phase 2 feasibility study were presented at the San Antonio Breast Cancer Symposium in December 2016 [20], and showed no benefit from the addition of gemcitabine. In view of this the trial (tnAcity study) will not proceed to a randomised phase III study. However there have been positive results in other solid tumours most notably pancreatic cancer[21]. Perhaps the addition of a fourth drug to three effective drugs is simply not going to improve DFS and OS in early breast cancer. The companion neoadjuvant trial (Neo-*tAnGo*)

also showed no increase in pathological complete response (pCR) rate with the addition of gemcitabine and no improvement in DFS and OS[22]. In addition, neither the neoadjuvant NSABP-B40[22,23] trial examining both capecitabine and gemcitabine added to paclitaxel, nor the adjuvant NSABP-B38[8] using AC-TG as one of the experimental arms, showed any improvement in pCR or DFS and OS.

Since gemcitabine, an anti-metabolite, is in the same class of drugs as capecitabine, recent data on that drug is of interest. The adjuvant TACT 2 trial demonstrated that capecitabine has some advantages over standard CMF following anthracycline-based treatment [submitted, January 2017], because of lower toxicity and better quality of life with no apparent loss of efficacy. However, when capecitabine was added to docetaxel, doxorubicin and cyclophosphamide in the FINNXX trial[23], there was no improvement in DFS and when capecitabine was substituted for cyclophosphamide in the GEICAM/2003-10 Study there was an increase in DFS events[24]. Nevertheless, there has been a recent renewal of interest, with the use of capecitabine after neoadjuvant chemotherapy in patients who had not achieved a pCR in the CREATE-X study [25]. The benefits of this adjuvant treatment, were significant and most marked in the TNBC group (296 patients: HR 0·58 [95% CI 0·39-0·87]). However, it is unlikely that gemcitabine could be used in this setting because of the significantly higher toxicity particularly myelosuppression.

Are there potential subgroups in *tAnGo* in which increased benefit from adjuvant gemcitabine could be further explored? Two groups identified are the ER-negative/ grade3, HER2 positive or negative subsets (Figure 4c and 4f), which also showed a possible benefit for pCR in Neo-*tAnGo*[22]. Recent preclinical research has reported compelling results of relevance to gemcitabine use in breast cancer[26], showing that mutant p53 (mtp53) harbouring cells are

highly sensitive to the cell killing effects of gemcitabine via inhibition of deoxycytidine kinase (dCK). In addition, dCK and/or p53 knockdown of these cells, abrogating the gain-of-function, conferred relative resistance to gemcitabine but not to cisplatin or doxorubicin. mtp53 status has been shown to be related to poor prognosis in breast cancer patients[27] and was recently studied in GEPAR SIXTO[28]. mtp53 was present in 297/450 (66%) patients, more frequently in TNBC (184/246 [74·8%]) compared to HER2-positive cancers (113/204 [55·4%] $p<0·0001$). As part of the *tAnGo* and Neo-*tAnGo* studies we have collected 80% of FFPE tumour samples for translational research and plan to explore this preclinical data further.

In summary, the *tAnGo*, Neo-*tAnGo*, NSABP-B38 and NSABP-B40 trials all demonstrate a lack of activity when gemcitabine is added to three potent neo/adjuvant breast cancer chemotherapy drugs. *tAnGo* was a large, rapidly recruiting trial with sufficient follow up to allow confidence in this result. Parallel translational science for *tAnGo* is ongoing, including tumour genomic analysis, pharmacogenetics[29,30] and inherited predisposition analyses[31]. Clinical trials datasets, painstakingly collected and analysed, clearly need to be published fully including all negative studies. The publication of a 'negative' trial is as important for patients and the clinical research community as the positive trial results which lead to licensing approvals or change of practice that are generally perceived as more valuable. The rejection of gemcitabine from standard adjuvant breast cancer treatment for lack of benefit, can be seen as a cost-effective result in terms of both financial and patient toxicity costs.

CONCLUSION

Gemcitabine in combination with paclitaxel after epirubicin and cyclophosphamide is not indicated in adjuvant breast cancer treatment with current biological characterisation. However, it seems likely that further improvements in the outcome of treatment for women with early breast cancer will depend on the development of targeted therapies, whose selective application is predicated on the biological heterogeneity of this disease.

Contributors

CP was responsible for the grant writing, conception, design, trial co-ordination, data analysis and interpretation; as well as result interpretation, manuscript drafting, patient recruitment, and Chair of the Trial steering Committee.

AMW was responsible for the grant writing, conception, design, trial co-ordination, data acquisition and interpretation; as well as result interpretation, manuscript drafting, patient recruitment, and sitting on the Trial steering Committee.

HE was responsible for the grant writing, conception, design, data analysis and interpretation; as well as result interpretation, manuscript drafting, patient recruitment, and sitting on the Trial steering Committee.

CC was responsible for the grant writing, conception, data acquisition and interpretation; as well as result interpretation, patient recruitment, and sitting on the Trial steering Committee.

DC was responsible for the conception, design, data and result interpretation; as well as manuscript drafting, patient recruitment, and sitting on the Trial steering Committee.

DD was responsible for the, design, trial co-ordination, data and result interpretation; as well as manuscript drafting, patient recruitment, and sitting on the Trial steering Committee.

DWR was responsible trial co-ordination, data acquisition, analysis and interpretation; as well as result interpretation, manuscript drafting and patient recruitment.

JD was responsible for the grant writing, conception, design, result interpretation, manuscript drafting, and sitting on the Trial steering Committee.

PC was responsible for the conception, design, data acquisition, result interpretation, patient recruitment, and sitting on the Trial steering Committee.

LH was responsible for design, data analysis and interpretation, result interpretation, manuscript drafting, and sitting on the Trial steering Committee.

HH was responsible for the grant writing, design, trial co-ordination, data acquisition and sitting on the Trial steering Committee.

JAC was responsible for the grant writing, conception design and sitting on the Trial steering Committee.

RC was responsible for design, manuscript drafting, patient recruitment and sitting on the Trial steering Committee.

RL was responsible for design, patient recruitment and sitting on the Trial steering Committee.

JPC was responsible for trial co-ordination, manuscript drafting and patient recruitment.

SB was responsible for trial co-ordination, data acquisition and manuscript drafting.

JA was responsible for the grant writing and patient recruitment.

AKW and JY were responsible for the trial co-ordination and data acquisition.

DMR and JK were responsible for the data acquisition and patient recruitment.

MM was responsible for the trial co-ordination.

EP was responsible data acquisition.

AG, AB, GW, KM, RA, SO and TH were responsible for patient recruitment.

All authors revised the manuscript, approved the final manuscript, and agreed on all aspects of the work.

Declaration of interests

AMW reports personal fees from Roche, Pfizer, and AstraZeneca, as well as non-financial support and travel support from Amgen, and travel support from Celgene; all outside the submitted work.

CP reports grants and non-financial support from both Eli Lilly and Bristol Myers Squibb, and grants from Pfizer, during the conduct of the study; CP also reports grants, personal fees and non-financial support from Eli Lilly, Pfizer and Bristol Myers Squibb, outside the submitted work.

DC reports time spent on an IDMC for a Lilly sponsored trial from Lilly, outside the submitted work.

DD has taken part in teaching, educational services and consultancy for which honoraria were paid by various pharmaceutical companies but not Eli Lilly.

HE reports unrestricted educational grants and free bevacixumab from Roche, and unrestricted educational grants from Sanofi-Aventis, all for the ARTemis Trial as well as Advisory Board honorarium from Pfizer. All outside the submitted work.

JAC reports honoraria and consulting fees received from Lilly Industries Ltd and Bristol-Myers Squibb Pharmaceuticals Ltd, at the time of planning this study.

JPC reports non-financial support from Bristol Myers Squibb, outside the submitted work.

KM reports personal fees from Roche Medical Meeting, and Novartis Advisory Board for Metastatic Breast Cancer, outside the submitted work.

RC reports grants from Bayer, Amgen and Celgene, to his institution outside the submitted work.

SB reports grants from Bristol-Myers Squibb Pharmaceuticals Ltd, and Lilly Industries Ltd., who provided free drug and an educational grant for the conduct of this study to the sponsor (her employer). SB also reports grants from Pharmacia (now Pfizer UK) who provided an

educational grant, for the conduct of this study to the Sponsor (her employer) during the conduct of the study. BMS, Lilly Industries and Pharmacia had no input into the design, conduct, or interpretation of the study results.

TH reports grants from Roche, during the conduct of the study.

AB, AG, AKW, CC, DMR, DWR, EP, GW, HH, JA, JD, JK, JY, LH, MM, PC, RA, RL, and SO have nothing to disclose.

and data managers funded via the NHS R&D departments (NIHR in England, Wales and N.Ireland; SCRN in Scotland; Government Research Funding in Eire).

tAnGo Investigators:

Trial Management Group: Christopher Poole (University Hospital, Coventry and Warwickshire); Helena Earl (Addenbrooke's Hospital); Louise Hiller and Janet Dunn (Warwick Clinical Trials Unit); Helen Howard (Leeds Institute of Clinical Trials Research); Jennie Young and Sarah Bowden (Cancer Research UK Clinical Trials Unit); Peter Harvey (formally of St. James's University Hospital); Andrew Stanley (formally of City Hospital, Birmingham); and David Rowlands (formally of New Cross Hospital).

Trials Steering Committee: members of the Trial Management Group (Christopher Poole; Helena Earl; Louise Hiller); John Bartlett (Ontario Institute for Cancer Research, Canada); Peter Barret- Lee (Velindre Hospital, UK); Carlos Caldas (University of Cambridge, UK); David Cameron (University of Edinburgh, UK); Peter Canney (The Beatson, UK); Robert Coleman (Weston Park Hospital, UK); David Dodwell, Tim Perren and Chris Twelves (St James' University Hospital, Leeds, UK); Ian Ellis (City Hospital, Nottingham, UK); Paul Ellis (King's College Hospital, UK); Robert Grieve (University Hospital, Coventry, UK); John Kennedy (St James's Hospital, Dublin, Ireland); Robert Leonard (Imperial College, London, UK); Michael Lind and Lynn Cawkwell (Castle Hill Hospital, UK); Janine Mansi (Guy's Hospital, UK); Susan O'Reilly (Clatterbridge Centre for Oncology, UK); Jyotsna Shrimanker (Royal Victoria Infirmary, UK); Jane Steven (University Hospital Birmingham NHS Foundation Trust, UK); Mark Verrill (Newcastle General Hospital, UK); Andrew Wardley (The Christie Hospital, UK).

Recruiting Sites and Investigators: Aberdeen Royal Infirmary, Aberdeen (n=21) Andrew Hutcheon; Addenbrooke's Hospital, Cambridge (n=57) Helena Earl, Karen McAdam;

Airedale General Hospital, Keighley (n=24) Michael Crawford; Alexandra Hospital, Redditch (n=1) Clive Irwin; Ayr Hospital, Ayr (n=12) Diana Ritchie; Basildon Hospital, Basildon (n=1) Colin Trask; Basingstoke and North Hampshire Hospital, Basingstoke (n=4) Sandra Tinkler; Beaumont Hospital, Dublin (n=5) Liam Grogan; Belfast City Hospital, Belfast (n=7) Sarah McKenna, Jacqueline Clarke; Birmingham Heartlands Hospital, Birmingham (n=2) Indrajit Fernando; Birmingham Priory Hospital, Birmingham (n=4) Christopher Poole, Daniel Rea; Bishop; Auckland General Hospital, Bishop Auckland (n=7) Nicholas Wadd; Bon Secours Hospital, Cork (n=2) Gordon Mullins; Bradford Royal Infirmary, Bradford (n=18) Chris Bradley, Chris Twelves; Bristol Haematology And Oncology Centre, Bristol (n=10) Christopher Price, Jeremy Braybrooke; Bronglais General Hospital, Aberystwyth (n=6) Alan Axford; Broomfield Hospital, Chelmsford (n=44) Neville Davidson; Burnley General Hospital, Burnley (n=4) Martin Hogg, Wiebke Appel; Calderdale Royal Hospital, Halifax (n=8) Jo Dent, Johnathan K Joffe; Charing Cross Hospital, London (n=17) C P Lowdell, Phillippa Riddle; Cheltenham General Hospital, Cheltenham (n=37) Sean Elyan, Kim Benstead, R Owen, Peter Jenkins, Radhika Counsell; Christie Hospital, Manchester (n=180) Andrew Wardley, Gregory Wilson, Richard Welch, A Loncaster, Peter Wilkinson; City Hospital, Birmingham (n=35) David Spooner, Daniel Rea; Clatterbridge Cancer Centre, Wirral (n=63) Susan M O'Reilly, Zafar Malik, Nicola Thorpe, Peter Clark; Cookridge Hospital, Leeds (n=49) David Dodwell, Sri Kumar, Fiona Roberts; Cork University Hospital, Cork (n=1) Oscar Breathnach; Countess of Chester Hospital, Chester (n=45) Joseph Maguire; County Hospital, Hereford, Hereford (n=3) Sean Elyan; Crosshouse Hospital, Kilmarnock (n=47) Diana Ritchie; Darlington Memorial Hospital, Darlington (n=11) Peter Hardman; Derbyshire Royal Infirmary, Derby (n=21) David Otim-Oyet, Pamela L Woodings; Derriford Hospital, Plymouth (n=8) Nigel Bailey, Stephen Kelly; Diana Princess of Wales Hospital, Grimsby (n=10) Paul Mack; Dorset County Hospital, Dorchester (n=26) Susan Dean, Perric Crellin; Dumfries and

Galloway Royal Infirmary, Dumfries (n=7) Ian Kunkler; Essex County Hospital, Colchester (n=42) Philip Murray; Friarage Hospital, Northallerton (n=5) J Van Der Voet; Furness General Hospital, Barrow-in-Furness (n=9) Gerraldine Ep Skailes; Glasgow Royal Infirmary, Glasgow (n=17) Ghislane Fraser, David Dunlop, Mike Soukop; Good Hope Hospital, Sutton Coldfield (n=14) Andrea Stevens; Guy's Hospital, London (n=40) Paul A Ellis, David Miles; Hairmyres Hospital, Hairmyres (n=23) Hosney Yosef; Halton General Hospital, Runcorn (n=3) Susan O'Reilly; Hinchingbrooke Hospital, Huntingdon (n=27) Simon G Russell, David Gilligan; Homerton University Hospital, London (n=1) Sarah Slater; Huddersfield Royal Infirmary, Huddersfield (n=28) Johnathan Joffe, Jo Dent, Barbara Crosse; King George Hospital, Ilford (n=29) Eliot Sims, David Propper; King's College Hospital, London (n=17) Paul Ellis; Luton and Dunstable Hospital, Luton (n=2) Andreas Makris; Macclesfield District General Hospital, Macclesfield (n=5) R Hunter; Manor Hospital, Walsall (n=9) Indrajit N Fernando; Middlesex Hospital, London (n=15) Robert C Stein; Milton Keynes General Hospital, Milton Keynes (n=5) Jill A Stewart; Mount Vernon Hospital, Northwood (n=40) Peter Ostler, Mark Harrison, Richard Ashford, Marcia R Hall, Andreas Makris, Jane Maher; Musgrove Park Hospital, Taunton (n=2) John Graham, Olivera Frim; New Cross Hospital, Wolverhampton (n=13) Mark Churn, Rakesh Mehra; Newcastle General Hospital, Newcastle upon Tyne (n=53) Mark Verrill, Kate Sumpter; Newham University Hospital, London (n=3) C Gallagher; North Devon District Hospital, Barnstaple (n=19) Mark P Napier; North Middlesex Hospital, London (n=24) Stephen Karp, F Neave, Jacqueline Newby; North Tyneside General Hospital, North Shields (n=13) Anthony N Branson; Northampton General Hospital, Northampton (n=55) Roy Mathew, Craig H MacMillan, A Stewart, Leo Houghton; Oldchurch Hospital, Romford (n=38) Eliot Sims, Mary Quigley; Peterborough District Hospital, Peterborough (n=38) Karen McAdam; Poole Hospital, Poole (n=51) Darcy Goode, Susan Dean Hickish; Princess Royal Hospital, Hull (n=34) Sunil Upadhyay, A Chatuverdi, Michael Lind; Queen Elizabeth Hospital,

Gateshead (n=24) Anthony Branson, Helen Lucraft; Queen Elizabeth Hospital, King's Lynn (n=23) Athar Ahmad; Queen Elizabeth Hospital, London (n=1) Bruce Bryant; Queen's Hospital, Burton upon Trent (n=6) A D Chetiyawardana, Prabir Chakraborti; Raigmore Hospital, Inverness (n=4) David Whillis, Carol MacGregor; Royal Albert Edward Infirmary, Wigan (n=38) Gregory Wilson; Royal Bournemouth Hospital, Bournemouth (n=53) Tamas Hickish; Royal Devon and Exeter Hospital, Exeter (n=60) Andrew Gordon Goodman, Anne Hong, Peter Bliss; Royal Free Hospital, London (n=28) Alison Jones; Royal Hampshire County Hospital, Winchester (n=19) Virginia L Hall; Royal Lancaster Infirmary, Lancaster (n=13) M B McIllmurray, Gerraldine Ep Skailes; Royal Liverpool University Hospital, Liverpool (n=34) Susan M O'Reilly, Nicola Thorpe, Royal Marsden Hospital London, London (n=22) Ian Smith, Steve Johnston; Royal Marsden Hospital Sutton, Sutton (n=25) Steve Johnston, Ian E Smith; Royal Preston Hospital, Preston (n=22) E Young, Martin Hogg; Royal Shrewsbury Hospital, Shrewsbury (n=72) Rajiv K Agrawal; Royal Surrey County Hospital, Guildford (n=52) Stephen Houston, Robert Laing, Anthony Neal, David Bloomfield; Royal Sussex County Hospital, Brighton (n=4) George Deutsch; Russells Hall Hospital, Dudley (n=15) Rozenn Allerton, Salisbury District Hospital, Salisbury (n=19) Kate Gregory; Sandwell General Hospital, West Bromwich (n=14) David Spooner; Singleton Hospital, Swansea (n=103) Robert Leonard, John Wagstaff, Gianfilippo Bertelli; South Infirmary - Victoria Hospital, Cork (n=3) Seamus O'Reilly; South Tyneside District Hospital, South Shields (n=29) Joseph M Bozzino, Waldemar Szpak; Southampton General Hospital, Southampton (n= 25) Peter Simmonds, Nicholas Murray, Andrew Last; Southend University Hospital, Westcliff-on-Sea (n=45) Anne Robinson, Colin Trask, Osama M Koriech; Southport and Formby District General Hospital, Southport (n=4) Khizar Hayat; St Bartholomew's Hospital, London (n=12) C Gallagher; St George's Hospital, London (n=44) Janine L Mansi; St James's Hospital, Dublin (n=9) M J Kennedy;St James's University Hospital, Leeds (n=38)

Timothy J Perren;St Margaret's Hospital, Epping (n=2) Julian Singer; St Mary's Hospital, Portsmouth (n=10) Caroline Archer, Tim Gulliford, J D Dubois; St Vincent's University Hospital, Dublin (n=27) J P Crown, David Fennelly; Sunderland Royal Hospital, Sunderland (n=13) Ujjall Mallick; The James Cook University Hospital, Middlesbrough (n=53) Alison Humphreys, Adrian J Rathmell, J Van Der Voet, Nicola Storey; The Queen Elizabeth Hospital, Birmingham (n=87) Christopher J Poole, Daniel W Rea, Indrajit N Fernando, Andrea Stevens, Talaat N Latief; Thornbury Hospital, Sheffield (n=8) I H Manifold; Torbay Hospital, Torquay (n=45) Peter Bliss, Andrew Goodman, Nigel Bailey; University College Hospital, Galway (n=11) MacCon Keane; University College London Hospital, London (n=2) Robert C Stein; University Hospital Aintree, Liverpool (n=5) C Penelope Schofield; Velindre Hospital, Cardiff (n=6) Peter Barrett-Lee; Victoria Hospital, Blackpool (n=7) Shabbir Susnerwala, Andrew Hindley; Walsgrave Hospital, Coventry (n=2) Robert J Grieve; Wansbeck General Hospital, Ashington (n=28) Waldemar Szpak, Anthony N Branson; Warrington Hospital, Warrington (n=25) Peter I Clark; Warwick Hospital, Warwick (n=1) David A Jones; West Suffolk Hospital, Bury St Edmunds (n=12) Margaret Moody; West Wales General Hospital, Carmarthen (n=3) Margaret F Wilkins; Western General Hospital, Edinburgh (n=101) Angela Bowman, David A Cameron; Western Infirmary, Glasgow (n=143) Peter Canney, Diana Ritchie, Chris Twelves, Ghislane Fraser; Weston General Hospital, Weston-super-Mare (n=4) Christopher Price; Weston Park Hospital, Sheffield (n=108) Robert Coleman, O P Purohit,I H Manifold, Matthew Hatton, K S Dunn, Bernadette Foran, S Ramakrishnan; Wexham Park Hospital, Slough (n=14) Marcia R Hall, Richard Ashford; Whipps Cross University Hospital, London (n=15) Chris Cottrill; Whiston Hospital, Prescot (n=12) Aileen Flavin; Whittington Hospital, London (n=15) Alison L Jones; Withybush General Hospital, Haverfordwest (n=15) Robert F Leonard; Withybush General Hospital,

Haverfordwest (n=5) Gianfilippo Bertelli; Worthing Hospital, Worthing (n=24) Sankha Mitra;

York Hospital, York (n=8) David Dodwell

FIGURE Legends

Figure 1: Trial Profile

Figure 2: Disease-free survival and overall survival by treatment group

Figure 3: Hazard rates over time

Figure 4: Disease-Free Survival and Overall Survival by Treatment, split by prognostic factors

Table 1: Patient Characteristics

		EC-GT (n=1570)		EC-T (n=1571)		Total (n=3141)	
		N	%	N	%	N	%
* Randomising Country	England	1282	81	1283	81	2565	81
	Scotland	189	12	185	12	374	12
	Wales	66	4	72	4	138	4
	Republic of Ireland	30	2	27	2	57	2
	Northern Ireland	3	1	4	1	7	1
* Age	≤50 years old	862	55	867	55	1729	55
	>50 years old	708	45	704	45	1412	45
* ER Status	Negative	692	44	686	44	1378	44
	Weakly-positive	168	11	197	12	365	12
	Positive	710	45	688	44	1398	44
PgR Status	Negative	695	44	703	45	1398	44
	Weakly-positive	181	12	165	11	346	11
	Positive	524	33	510	32	1034	33
	Unknown	170	11	193	12	363	12
* Nodal Status	Negative	364	23	362	23	726	23
	1-3 nodes positive	648	41	646	41	1294	41
	≥4 nodes positive	558	36	563	36	1121	36
* Radiotherapy planned	Yes	1412	90	1411	90	2823	90
	No	158	10	160	10	318	10
* HER2 status	+++	135	9	152	10	287	9
	Other (0, 1+, 2+)	1015	64	990	63	2005	64

	Unknown	420	27	429	27	849	27
ECOG performance status	0	1433	91	1464	93	2897	92
	1	135	8	106	6	241	7
	2	2	1	1	1	3	1
Menopausal Status	Pre	728	46	701	45	1429	45
	Peri	104	7	131	8	235	7
	Post	560	36	556	35	1116	36
	Bilateral Oophorectomy	14	1	12	1	26	1
	Hysterectomy	87	5	106	7	193	6
	Unknown	77	5	65	4	142	5
Definitive Surgery	Mastectomy	874	56	875	56	1749	56
	Breast Conserving Surgery	696	44	696	44	1392	44
Days from Definitive Surgery to Entry	N	1570		1571		3141	
	Median (IQR)	31 (24 – 40)		32 (25 – 41)		32 (25 – 40)	
	Range	6 – 76		-9 – 80		-9^ – 80	
Triple negatives	Yes	364	23	362	23	726	23
	No	1028	66	1031	66	2059	66
	Unknown	178	11	178	11	356	11
ER/PGR negatives	Yes	561	36	562	36	1123	36
	No	955	61	946	60	1901	60
	Unknown	54	3	63	4	117	4

* Stratification variables at randomisation

^ 2 patients were randomised prior to definitive surgery, after authorisation from the Chief Investigator

Table 2: Causes of death, and types and sites of relapse

	EC-GT (n=1570)		EC-T (n=1571)		Total (n=3141)	
	N	%	N	%	N	%
Deaths	459	29	455	29	914	29
Main cause of death Breast Cancer	389	85	403	88	792	86
Other cancer	22	5	17	4	39	4
Cardiovascular disease	9	2	8	2	17	2
Infection	5	1	5	1	10	1
Venous thromboembolism	4	1	4	1	8	1
Other	10	2	4	1	14	2
Unknown	20	4	14	3	34	4
Relapses	486	31	509	32	995	32
Type of Relapse * Local	122	8	149	9	271	9
Distant	454	29	458	29	912	29
Site of Local Relapse * Breast/Chest Wall	95	78	116	78	211	78
Axilla	28	23	36	25	64	24
Ipsilateral SCF	6	5	5	3	11	4
Unknown	1	1	-	-	1	<1
Site of Distant Relapse* Bone	226	50	199	43	425	47
Liver	171	38	170	37	341	37
Lung	114	25	119	26	233	26
Lymph node	68	15	69	15	137	15
Brain	58	13	73	16	131	14
Skin	7	2	8	2	15	2
Ovary	5	1	5	1	10	1

Other	30	7	28	6	58	6
Second Primaries	109	7	104	7	213	7
Breast	48	44	48	46	96	45
Non-breast	61	56	56	54	117	55
Deaths and Relapses	538	34	549	35	1087	35

*Some patients have multiple types of relapse (local and distant), and sites of relapse

Table 3: Disease-Free Survival according to treatment, patient and tumour characteristics

Variable	No. of patients*	No. of Events	p-value	DFS rates % (95% CI) 2 year	5 year	10 year
Overall	3141	1087		88 (86-89)	75 (73-76)	65 (63-67)
Treatment group			0·63			
EC-GT	1570	538		88 (87-90)	75 (73-77)	65 (63-68)
EC-T	1571	549		87 (85-88)	74 (72-76)	65 (62-67)
Number of nodes involved			<0·0001			
0	726	147		93 (91-95)	86 (84-89)	79 (76-82)
1-3	1294	378		91 (89-92)	79 (76-81)	71 (68-73)
4+	1121	562		80 (77-82)	62 (59-65)	50 (47-53)
Oestrogen-receptor status			<0·0001			
Negative	1378	513		82 (79-83)	69 (66-71)	62 (60-65)
Weakly-positive	365	130		86 (82-89)	74 (70-79)	64 (59-69)
Positive	1398	444		94 (92-95)	80 (78-82)	68 (66-71)
Progesterone-receptor status			<0·0001			
Negative	1398	517		83 (81-85)	69 (66-71)	63 (60-65)
Weakly-positive	346	129		87 (83-90)	74 (69-78)	62 (57-67)
Positive	1034	312		94 (93-96)	83 (80-85)	70 (67-72)
Radiotherapy planned			<0·0001			
Yes	2823	1012		87 (85-88)	73 (72-75)	64 (62-66)

No	318	75		95 (92-97)	86 (82-89)	76 (71-80)
Tumour size			<0·0001			
≤2cm	1122	280		92 (90-93)	82 (80-85)	75 (72-77)
>2 and ≤5cm	1663	648		86 (84-88)	71 (69-73)	61 (58-63)
>5cm	266	133		77 (71-81)	61 (55-67)	49 (43-55)
Tumour Grade			<0·0001			
Well differentiated	49	10		100 (100-100)	94 (82-98)	79 (65-88)
Moderately differentiated	1141	355		92 (91-94)	80 (78-83)	69 (66-71)
Poorly differentiated	1948	722		84 (83-86)	71 (69-73)	63 (60-65)
Vascular/Lymphatic invasion			<0·0001			
Reported	1836	752		84 (82-85)	69 (67-71)	59 (57-61)
Unreported	1303	335		93 (91-94)	82 (80-84)	74 (72-76)
Surgery			<0·0001			
Mastectomy	1749	673		86 (84-87)	72 (69-74)	61 (59-64)
Breast Conserving Surgery	1392	414		90 (88-91)	78 (76-80)	70 (67-72)
ECOG performance status			0·004			
0	2897	983		88 (87-89)	75 (74-77)	66 (64-68)
1 or 2	244	104		84 (79-88)	67 (60-72)	57 (51-63)
HER-2 status			0·0026			
+++	287	119		82 (77-86)	66 (60-71)	58 (52-64)
Other	2005	679		88 (87-90)	76 (74-78)	66 (64-68)

Age				0·80			
≤50 years old	1729	596			87 (85-88)	73 (71-75)	65 (63-67)
>50 years old	1412	491			88 (86-90)	76 (74-78)	65 (63-68)
Menopausal status				0·86			
Pre	1429	489			88 (86-89)	74 (71-76)	65 (63-68)
Peri	235	80			89 (84-92)	76 (70-81)	66 (59-71)
Post	1116	397			87 (85-89)	76 (73-78)	64 (61-67)
Triple negatives				0·01			
Yes	726	266			81 (78-84)	69 (65-72)	63 (59-66)
No	2059	695			91 (89-92)	77 (75-79)	66 (64-68)
ER/PGR negative				0·0008			
Yes	1123	412			81 (79-84)	69 (66-71)	63 (60-66)
No	1901	626			92 (90-93)	78 (77-80)	67 (65-69)

* Patients with missing data for a given variable were excluded from the analysis of that variable

Table 4: Maximum reported grades of Adverse Effects during treatment by 3132 patients

	EC-GT (n=1565)				EC-T (n=1567)			
	1	2	3	4	1	2	3	4
Neutropenia	171 (11%)	226 (14%)	**323 (21%)**	204 (13%)	174 (11%)	190 (12%)	**212 (14%)**	200 (13%)
Myalgia/arthralgia	513 (33%)	627 (40%)	200 (13%)	7 (<1%)	501 (32%)	646 (41%)	175 (11%)	11 (1%)
Fatigue	395 (25%)	**859 (55%)**	**198 (13%)**	9 (1%)	508 (32%)	**779 (50%)**	**140 (9%)**	12 (1%)
Infection	197 (13%)	381 (24%)	**194 (12%)**	8 (1%)	214 (14%)	387 (25%)	**131 (8%)**	10 (1%)
Vomiting	399 (26%)	**387 (25%)**	**134 (9%)**	9 (1%)	413 (26%)	**323 (21%)**	**101 (6%)**	7 (1%)
Nausea	611 (39%)	660 (42%)	132 (8%)	-	694 (44%)	561 (36%)	102 (7%)	-
Neuro-sensory	756 (48%)	377 (24%)	83 (5%)	2 (<1%)	809 (52%)	365 (23%)	66 (4%)	3 (<1%)
Fever	237 (15%)	95 (6%)	69 (4%)	1 (<1%)	171 (11%)	64 (4%)	46 (3%)	3 (<1%)
Diarrhoea	465 (30%)	183 (12%)	43 (3%)	1 (<1%)	453 (29%)	154 (10%)	29 (2%)	0
Constipation	597 (38%)	489 (31%)	41 (3%)	1 (<1%)	605 (39%)	494 (32%)	24 (2%)	0
Dyspnoea	-	485 (31%)	37 (2%)	3 (<1%)	-	423 (27%)	30 (2%)	3 (<1%)
Stomatitis	669 (43%)	450 (29%)	31 (2%)	0	692 (44%)	403 (26%)	25 (2%)	0
Anaemia	565 (36%)	**308 (20%)**	27 (2%)	1 (<1%)	510 (33%)	**119 (8%)**	11 (1%)	3 (<1%)
Skin	535 (34%)	168 (11%)	25 (2%)	0	503 (32%)	148 (9%)	20 (1%)	1 (<1%)
DVT	-	4 (<1%)	17 (1%)	3 (<1%)	-	2 (<1%)	9 (1%)	3 (<1%)
Thrombocytopenia	110 (7%)	20 (1%)	14 (1%)	4 (<1%)	57 (4%)	18 (1%)	7 (<1%)	2 (<1%)
Cough	455 (29%)	115 (7%)	10 (1%)	-	432 (28%)	94 (6%)	4 (<1%)	-
Superficial Thrombophlebitis	-	367 (23%)	7 (<1%)	0	-	329 (21%)	1 (<1%)	1 (<1%)
Alopecia	44 (3%)	1481 (95%)	-	-	37 (2%)	1490 (95%)	-	-

REFERENCES

1.   Early Breast Cancer Trialists' Collaborative Group. Polychemotherapy for early breast cancer: an overview of the randomised trials. Early Breast Cancer Trialists' Collaborative Group. *Lancet* 1998; **352**(9132): 930-42.

2.   Poole CJ, Earl HM, Hiller L, et al. Epirubicin and cyclophosphamide, methotrexate, and fluorouracil as adjuvant therapy for early breast cancer. *N Engl J Med* 2006; **355**(18): 1851-62.

3.   Levine MN, Pritchard KI, Bramwell VH, Shepherd LE, Tu D, Paul N. Randomized trial comparing cyclophosphamide, epirubicin, and fluorouracil with cyclophosphamide, methotrexate, and fluorouracil in premenopausal women with node-positive breast cancer: update of National Cancer Institute of Canada Clinical Trials Group Trial MA5. *J Clin Oncol* 2005; **23**(22): 5166-70.

4.   Coombes RC, Bliss JM, Wils J, et al. Adjuvant cyclophosphamide, methotrexate, and fluorouracil versus fluorouracil, epirubicin, and cyclophosphamide chemotherapy in premenopausal women with axillary node-positive operable breast cancer: results of a randomized trial. The International Collaborative Cancer Group. *J Clin Oncol* 1996; **14**(1): 35-45.

5.   Henderson IC, Berry DA, Demetri GD, et al. Improved outcomes from adding sequential Paclitaxel but not from escalating Doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. *J Clin Oncol* 2003; **21**(6): 976-83.

6.   Mamounas EP, Bryant J, Lembersky B, et al. Paclitaxel after doxorubicin plus cyclophosphamide as adjuvant chemotherapy for node-positive breast cancer: results from NSABP B-28. *J Clin Oncol* 2005; **23**(16): 3686-96.

7.   Early Breast Cancer Trialists' Collaborative G, Peto R, Davies C, et al. Comparisons between different polychemotherapy regimens for early breast cancer: meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet* 2012; **379**(9814): 432-44.

8.   Swain SM, Tang G, Geyer CE, Jr., et al. Definitive results of a phase III adjuvant trial comparing three chemotherapy regimens in women with operable, node-positive breast cancer: the NSABP B-38 trial. *J Clin Oncol* 2013; **31**(26): 3197-204.

9.   Earl HM, Vallier AL, Hiller L, et al. Effects of the addition of gemcitabine, and paclitaxel-first sequencing, in neoadjuvant sequential epirubicin, cyclophosphamide, and paclitaxel for women with high-risk early breast cancer (Neo-tAnGo): an open-label, 2x2 factorial randomised phase 3 trial. *Lancet Oncol* 2014; **15**(2): 201-12.

10.   Wardley AM, Hiller L, Howard HC, et al. tAnGo: a randomised phase III trial of gemcitabine in paclitaxel-containing, epirubicin/cyclophosphamide-based, adjuvant chemotherapy for early breast cancer: a prospective pulmonary, cardiac and hepatic function evaluation. *Br J Cancer* 2008; **99**(4): 597-603.

11.   Poole CJ, Hiller L, Howard HC, et al. tAnGo: A randomized phase III trial of gemcitabine (gem) in paclitaxel-containing, epirubicin/cyclophosphamide-based, adjuvant chemotherapy (CT) for women with early-stage breast cancer (EBC). *J Clin Oncol* 2008; **26**(15): 506-.

12.   Goldhirsch A, Gelber RD, Piccart-Gebhart MJ, et al. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): an open-label, randomised controlled trial. *Lancet* 2013; **382**(9897): 1021-8.

13.   Earl HM, Hiller L, Dunn JA, et al. NEAT: National Epirubicin Adjuvant Trial--toxicity, delivered dose intensity and quality of life. *Br J Cancer* 2008; **99**(8): 1226-31.

14.   Pocock SJ. Interim analyses for randomized clinical trials: the group sequential approach. *Biometrics* 1982; **38**(1): 153-62.

15.   Lan KK, Wittes J. The B-value: a tool for monitoring data. *Biometrics* 1988; **44**(2): 579-85.

16.   Earl HM. Reporting of adjuvant breast cancer trials: when is the right time? *J Clin Oncol* 2012; **30**(1): 1-2.

17.   Albain KS, Nag SM, Calderillo-Ruiz G, et al. Gemcitabine plus Paclitaxel versus Paclitaxel monotherapy in patients with metastatic breast cancer and prior anthracycline treatment. *J Clin Oncol* 2008; **26**(24): 3950-7.

18.   Hu XC, Zhang J, Xu BH, et al. Cisplatin plus gemcitabine versus paclitaxel plus gemcitabine as first-line therapy for metastatic triple-negative breast cancer (CBCSG006): a randomised, open-label, multicentre, phase 3 trial. *Lancet Oncol* 2015; **16**(4): 436-46.

19.   Yardley DA, Brufsky A, Coleman RE, et al. Phase II/III weekly nab-paclitaxel plus gemcitabine or carboplatin versus gemcitabine/carboplatin as first-line treatment of patients with metastatic triple-negative breast cancer (the tnAcity study): study protocol for a randomized controlled trial. *Trials* 2015; **16**: 575.

20.   Yardley D, Coleman R, Conte P. nab-paclitaxel + carboplatin or gemcitabine vs gemcitabine/carboplatin as first-line treatment for patients with triple-negative metastatic breast cancer: Results from the randomized phase 2 portion of the tnAcity trial. *Presented at: 2016 San Antonio Breast Cancer Symposium* 2016; **Abstract P5-15-03**.

21.   Von Hoff DD, Ervin T, Arena FP, et al. Increased survival in pancreatic cancer with nab-paclitaxel plus gemcitabine. *N Engl J Med* 2013; **369**(18): 1691-703.

22.   Earl HM, Cameron DA, Miles D, et al. PERSEPHONE: Duration of trastuzumab with chemotherapy in women with HER2-positive early breast cancer—Six versus twelve months. *J Clin Oncol* 2014; **32**(5s).

23.   Joensuu H, Kellokumpu-Lehtinen PL, Huovinen R, et al. Adjuvant capecitabine, docetaxel, cyclophosphamide, and epirubicin for early breast cancer: final analysis of the randomized FinXX trial. *J Clin Oncol* 2012; **30**(1): 11-8.

24.   Martin M, Ruiz Simon A, Ruiz Borrego M, et al. Epirubicin Plus Cyclophosphamide Followed by Docetaxel Versus Epirubicin Plus Docetaxel Followed by Capecitabine As Adjuvant Therapy for Node-Positive Early Breast Cancer: Results From the GEICAM/2003-10 Study. *J Clin Oncol* 2015; **33**(32): 3788-95.

25.   Toi M, Lee S-J, Lee E, et al. Abstract S1-07: A phase III trial of adjuvant capecitabine in breast cancer patients with HER2-negative pathologic residual invasive disease after neoadjuvant chemotherapy (CREATE-X, JBCRG-04). *Cancer Res* 2016; **76**(4 Supplement): S1-07-S1-.

26.   Kollareddy M, Dimitrova E, Vallabhaneni KC, et al. Regulation of nucleotide metabolism by mutant p53 contributes to its gain-of-function activities. *Nature communications* 2015; **6**: 7389.

27.   Pharoah PD, Day NE, Caldas C. Somatic mutations in the p53 gene and prognosis in breast cancer: a meta-analysis. *Br J Cancer* 1999; **80**(12): 1968-73.

28.   Darb-Esfahani S, Denkert C, Stenzinger A, et al. Role of TP53 mutations in triple negative and HER2-positive breast cancer treated with neoadjuvant anthracycline/taxane-based chemotherapy. *Oncotarget* 2016; **7**(42): 67686-98.

29.   Abraham JE, Guo Q, Dorling L, et al. Replication of genetic polymorphisms reported to be associated with taxane-related sensory neuropathy in patients with early breast cancer treated with Paclitaxel. *Clin Cancer Res* 2014; **20**(9): 2466-75.

30.   Abraham JE, Hiller L, Dorling L, et al. A nested cohort study of 6,248 early breast cancer patients treated in neoadjuvant and adjuvant chemotherapy trials investigating the prognostic value of chemotherapy-related toxicities. *BMC Med* 2015; **13**: 306.

31.   Guo Q, Schmidt MK, Kraft P, et al. Identification of novel genetic markers of breast cancer survival. *J Natl Cancer Inst* 2015; **107**(5).