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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, 2×2 factorial randomised phase 3 trial

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Summary

Background Anthracyclines and taxanes have been the standard neoadjuvant chemotherapies for breast cancer in the past decade. We aimed to assess safety and efficacy of the addition of gemcitabine to accelerated paclitaxel with epirubicin and cyclophosphamide, and also the effect of sequencing the blocks of epirubicin and cyclophosphamide and paclitaxel (with or without gemcitabine).

Methods In our randomised, open-label, 2×2 factorial phase 3 trial (Neo-tAnGo), we enrolled women (aged >18 years) with newly diagnosed breast cancer (tumour size >20 mm) at 57 centres in the UK. Patients were randomly assigned via a central randomisation procedure to epirubicin and cyclophosphamide then paclitaxel (with or without gemcitabine) or paclitaxel (with or without gemcitabine) then epirubicin and cyclophosphamide. Four cycles of each component were given. The primary endpoint was pathological complete response (pCR), defined as absence of invasive cancer in the breast and axillary lymph nodes. This study is registered with EudraCT (2004-002356-34), ISRCTN (78234870), and ClinicalTrials.gov (NCT00070278).

Findings Between Jan 18, 2005, and Sept 28, 2007, we randomly allocated 831 participants; 207 received epirubicin and cyclophosphamide then paclitaxel; 208 were given paclitaxel then epirubicin and cyclophosphamide; 208 had epirubicin and cyclophosphamide followed by paclitaxel and gemcitabine; and 208 received paclitaxel and gemcitabine then epirubicin and cyclophosphamide. 828 patients were eligible for analysis. Median follow-up was 47 months (IQR 37–51). 207 (25%) patients had inflammatory or locally advanced disease, 169 (20%) patients had tumours larger than 50 mm, 413 (50%) patients had clinical involvement of axillary nodes, 276 (33%) patients had oestrogen receptor (ER)-negative disease, and 191 (27%) patients had HER2-positive disease. Addition of gemcitabine did not increase pCR: 70 (17%, 95% CI 14–21) of 404 patients in the epirubicin and cyclophosphamide then paclitaxel group achieved pCR compared with 71 (17%, 14–21) of 408 patients who received additional gemcitabine (p=0·98). Receipt of a taxane before anthracycline was associated with improved pCR: 82 (20%, 95% CI 16–24) of 406 patients who received paclitaxel with or without gemcitabine followed by epirubicin and cyclophosphamide achieved pCR compared with 59 (15%, 11–18) of 406 patients who received epirubicin and cyclophosphamide first (p=0·03). Grade 3 toxicities were reported at expected levels: 173 (21%) of 812 patients who received treatment and had full treatment details had grade 3 neutropenia, 66 (8%) had infection, 41 (5%) had fatigue, 41 (5%) had muscle and joint pains, 37 (5%) had nausea, 36 (4%) had vomiting, 34 (4%) had neuropathy, 23 (3%) had transaminitis, 16 (2%) had acute hypersensitivity, and 20 (2%) had a rash. 86 (11%) patients had grade 4 neutropenia and 3 (<1%) had grade 4 infection.

Interpretation Although addition of gemcitabine to paclitaxel and epirubicin and cyclophosphamide chemotherapy does not improve pCR, sequencing chemotherapy so that taxanes are received before anthracyclines could improve pCR in standard neoadjuvant chemotherapy for breast cancer.

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Introduction

Survival of patients with early breast cancer has improved substantially in the past 20 years.[1] However, incidence of breast cancer has increased and continues to be a major health problem. The Early Breast Cancer Trialists Collaborative Group overview analyses,[2,3] and individual trial data have shown benefit for adjuvant anthracyclines[4–6] and taxane-containing chemotherapy.[7–9]

Progress made through large adjuvant randomised treatment trials has been relatively slow. Follow-up is necessarily prolonged to meet the prespecified event-rate criteria for disease-free survival (DFS) and overall

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survival analyses, as defined in statistical analysis plans.[10] However, in the neoadjuvant setting trials can report the primary endpoint of pathological complete response (pCR) more rapidly than their adjuvant counterparts. Broad acceptance of the long-term validity of neoadjuvant trials awaits formal comparison with data acquired from parallel, conventional adjuvant studies. tAnGo[11] and Neo-tAnGo were designed to provide this cross reference, because both trials investigated the addition of gemcitabine to standard chemotherapy, although the sequence of chemotherapy was not addressed in tAnGo.

Neo-tAnGo was a randomised phase 3 neoadjuvant trial that aimed to assess benefits of addition of gemcitabine to accelerated paclitaxel with epirubicin and cyclophosphamide, and also the effect of sequencing of epirubicin and cyclophosphamide, and paclitaxel (with and without gemcitabine) blocks. Gemcitabine is an effective drug in metastatic disease[12,13] and other neoadjuvant or adjuvant trials have addressed similar questions for antimetabolites, with gemcitabine[14,15] or oral capecitabine.[16] Neo-tAnGo was designed and started before the introduction of adjuvant trastuzumab for HER2-positive disease.

Methods

Study design and participants

In the Neo-tAnGo phase 3 randomised trial, we used a 2×2 factorial design to address both the role of gemcitabine in a sequential neoadjuvant chemotherapy regimen of epirubicin and cyclophosphamide and paclitaxel, and also the sequence of administration of these treatment components, in terms of short-term and long-term outcomes in women presenting with early breast cancer.

We enrolled women aged older than 18 years with a histological diagnosis of early invasive breast cancer, with a radiological tumour size of more than 20 mm with or without axillary involvement. Women were enrolled at 57 sites (NCRN Cancer Centres and Cancer Units) in the UK. Women with inflammatory cancer, T4 tumours with direct extension to the chest wall or skin, and ipsilateral supraclavicular lymph-node involvement were eligible with any size of primary tumour. We regarded hormone receptor status (oestrogen [ER] and progesterone [PR]) as positive when Allred score was 3 or higher. HER2 status was regarded as positive when immunohistochemistry was 3+, or 2+ with evidence of amplification of the HER2 gene on fluorescence in-situ hybridisation. Other eligibility criteria were adequate cardiac function, no myocardial infarction during the previous 6 months, adequate bone marrow, hepatic, and renal function, and appropriate ECOG performance status (0–2). No previous exposure to chemotherapy, radiotherapy or endocrine therapy was allowed. Full eligibility criteria can be found in the trial protocol and in the appendix. Patients provided written informed consent.

Neo-tAnGo was an investigator designed and led trial, which was granted Clinical Trials Authorisation from the Medicines and Healthcare products Regulatory Agency (MHRA) on June 17, 2004, and approved by the multicentre research ethics committee nationally on Nov 1, 2004, and subsequently the local research ethics committees at all participating centres. The study was undertaken by the UK National Cancer Research Institute (NCRI).

Randomisation and masking

In our open-label trial, eligible participants were randomly allocated 1:1:1:1 to receive epirubicin and cyclophosphamide followed by paclitaxel, paclitaxel followed by epirubicin and cyclophosphamide, epirubicin and cyclophosphamide followed by paclitaxel and gemcitabine, or paclitaxel and gemcitabine followed by epirubicin and cyclophosphamide. The paclitaxel (with or without gemcitabine) was accelerated. Stratification by minimisation was undertaken by age (≤50 years and >50 years), ER status (positive and negative), primary tumour size (≤5 cm and >5 cm), clinical involvement of axillary nodes, and inflammatory or locally advanced disease. We intended chemotherapy to start within 4 weeks of randomisation. Chemotherapy regimens used were epirubicin 90 mg/m² and cyclophosphamide 600 mg/m² once every 21 days, and paclitaxel 175 mg/m² with or without gemcitabine 2000 mg/m² once every 14 days. Each treatment component was given for four cycles per patient. Treatment allocations were made by telephoning the Cancer Research UK Trials Unit (Birmingham, UK), who used their central computerised minimisation procedure to generate the patients' random allocation.

Procedures

Our primary endpoint was pCR, defined as absence of invasive breast cancer in the breast and axillary lymph nodes, after neoadjuvant chemotherapy. A two-reader review of pathology reports was undertaken, masked to treatment group, by the chief investigator (HME) and the study pathologist (EP), for patients who had surgery. A detailed analysis of this review process has been published elsewhere.[17] Residual non-invasive ductal carcinoma in situ was allowed.

Secondary endpoints reported here include DFS and overall survival. We assessed adverse events for each chemotherapy cycle according to Common Terminology Criteria for Adverse Events (CTCAE) grade. We also recorded use of growth factor support (usually granulocyte colony stimulating factor [GCSF]).

The maximum permitted dose delay or interruption was 4 weeks to allow recovery from severe toxicity or for unscheduled procedures (eg, emergency surgery).

If neutropenic fever or sepsis occurred after a cycle of chemotherapy, the next cycle was delayed until the absolute neutrophil count was at least $1{\cdot}0\times10^9$ 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 100×10^9 platelets per L and was reduced to 80%, maintaining this dose reduction for subsequent cycles. Depending on sequence allocation, cycles from the next block of treatment were commenced at full protocol dose, and permitted delays and reductions were made as necessary. Primary prophylaxis with GCSF was not provided with either epirubicin and cyclophosphamide or accelerated 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.

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, paclitaxel and gemcitabine were stopped. If fewer than four cycles of paclitaxel (with or without gemcitabine) had been given, additional epirubicin and cyclophosphamide cycles were allowed up to a maximum

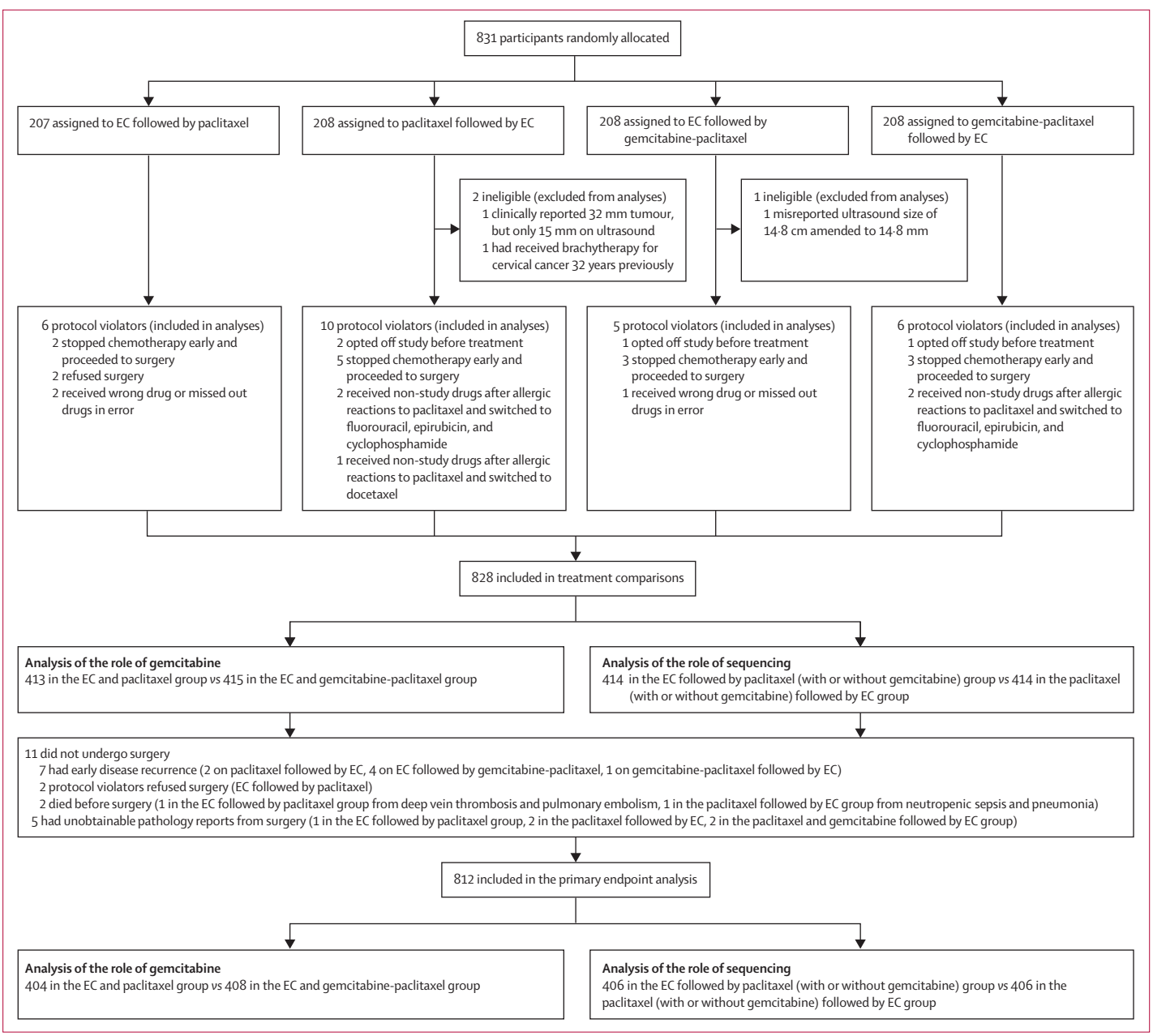

Figure 1: **Trial profile**
EC=epirubicin and cyclophosphamide.

	Component analysis		Sequencing analysis		Overall (n=828)
	EC and paclitaxel (n=413)	EC and paclitaxel-gemcitabine (n=415)	EC followed by paclitaxel with or without gemcitabine (n=414)	Paclitaxel with or without gemcitabine followed by EC (n=414)	
Demographics					
Age, years*					
≤50	261 (63%)	261 (63%)	260 (63%)	262 (63%)	522 (63%)
>50	152 (37%)	154 (37%)	154 (37%)	152 (37%)	306 (37%)
ER status*†					
Negative	137 (33%)	139 (33%)	139 (34%)	137 (33%)	276 (33%)
Positive	276 (67%)	276 (67%)	275 (66%)	277 (67%)	552 (67%)
PR status† (75% known)					
Negative	154 (49%)	152 (49%)	144 (47%)	162 (51%)	306 (49%)
Positive	158 (51%)	158 (51%)	160 (53%)	156 (49%)	316 (51%)
HER2 status‡ (85% known)					
Negative	257 (73%)	256 (73%)	251 (72%)	262 (74%)	513 (73%)
Positive	94 (27%)	97 (27%)	97 (28%)	94 (26%)	191 (27%)
Tumour size*					
≤50 mm	330 (80%)	329 (79%)	332 (80%)	327 (79%)	659 (80%)
>50 mm	83 (20%)	86 (21%)	82 (20%)	87 (21%)	169 (20%)
Clinical involvement of axillary nodes*					
No	208 (50%)	207 (50%)	208 (50%)	207 (50%)	415 (50%)
Yes	205 (50%)	208 (50%)	206 (50%)	207 (50%)	413 (50%)
Inflammatory or locally advanced disease*					
No	309 (75%)	312 (75%)	309 (75%)	312 (75%)	621 (75%)
Yes§	104 (25%)	103 (25%)	105 (25%)	102 (25%)	207 (25%)
Inflammatory disease	47 (45%)	55 (53%)	55 (52%)	47 (46%)	102 (49%)
Advanced disease	76 (73%)	69 (67%)	71 (68%)	74 (73%)	145 (70%)
Menopausal status					
Premenopausal	235 (57%)	235 (57%)	235 (57%)	235 (57%)	470 (57%)
Perimenopausal	26 (6%)	19 (5%)	22 (5%)	23 (6%)	45 (5%)
Postmenopausal	107 (26%)	114 (27%)	114 (28%)	107 (26%)	221 (27%)
Bilateral oophorectomy	4 (1%)	0 (0%)	1 (<1%)	3 (1%)	4 (<1%)
Hysterectomy	15 (4%)	23 (6%)	18 (4%)	20 (5%)	38 (5%)
Not known	26 (6%)	24 (6%)	24 (6%)	26 (6%)	50 (6%)
Number of invasive tumours					
1	236 (57%)	236 (57%)	243 (59%)	229 (55%)	472 (57%)
≥2	172 (42%)	176 (42%)	165 (40%)	183 (44%)	348 (42%)
Not known	5 (1%)	3 (1%)	6 (1%)	2 (1%)	8 (1%)
Details of largest breast tumour					
Tumour type¶					
Ductal or no special type	325 (79%)	325 (78%)	315 (76%)	335 (81%)	650 (79%)
Lobular	35 (8%)	41 (10%)	38 (9%)	38 (9%)	76 (9%)
Tubular or cribform	6 (1%)	4 (1%)	6 (1%)	4 (1%)	10 (1%)
Mucinous	2 (<1%)	4 (1%)	3 (1%)	3 (1%)	6 (1%)
Medullary	1 (<1%)	2 (<1%)	1 (<1%)	2 (<1%)	3 (1%)
Other	20 (5%)	18 (4%)	24 (6%)	14 (3%)	38 (5%)
Not known	40 (10%)	46 (11%)	43 (10%)	43 (10%)	86 (10%)
Tumour grade					
1 (well differentiated)	13 (3%)	8 (2%)	11 (3%)	10 (2%)	21 (3%)
2 (moderately differentiated)	123 (30%)	122 (29%)	130 (31%)	115 (28%)	245 (30%)
3 (poorly differentiated)	158 (38%)	172 (41%)	159 (38%)	171 (41%)	330 (40%)
Not known	119 (29%)	113 (27%)	114 (28%)	118 (29%)	232 (28%)

(Continues on next page)

	Component analysis		Sequencing analysis		Overall (n=828)
	EC and paclitaxel (n=413)	EC and paclitaxel-gemcitabine (n=415)	EC followed by paclitaxel with or without gemcitabine (n=414)	Paclitaxel with or without gemcitabine followed by EC (n=414)	
(Continued from previous page)					
Clinical characteristics¶					
Nipple retraction	40 (10%)	44 (11%)	35 (8%)	49 (12%)	84 (10%)
Skin infiltration	15 (4%)	16 (4%)	17 (4%)	14 (3%)	31 (4%)
Peau d'orange	26 (6%)	31 (7%)	30 (7%)	27 (7%)	57 (7%)
Redness	28 (7%)	27 (7%)	30 (7%)	25 (6%)	55 (7%)
Oedema	20 (5%)	26 (6%)	26 (6%)	20 (5%)	46 (6%)
Other	52 (13%)	50 (12%)	45 (11%)	57 (14%)	102 (12%)
None of the above	186 (45%)	186 (45%)	192 (46%)	180 (43%)	372 (45%)
Not known	79 (19%)	83 (20%)	80 (19%)	83 (20%)	162 (20%)
DCIS associated with tumour					
No	186 (45%)	183 (44%)	186 (45%)	183 (44%)	369 (45%)
Yes	110 (27%)	96 (23%)	112 (27%)	94 (23%)	206 (25%)
Not known	117 (28%)	136 (33%)	116 (28%)	137 (33%)	253 (31%)

EC=epirubicin-cyclophosphamide. ER=oestrogen receptor. PR=progesterone receptor. DCIS=ductal carcinoma in situ. *Stratification variables at randomisation. †ER and PR positive was a score of ≥3 on the 8 point Allred scale. ‡HER2-negative defined as immunohistochemistry score of 0–1, or 2, but fluorescence in-situ hybridisation (FISH) negative; HER2-positive defined as immunohistochemistry of ≥3 or ≥2 and FISH positive. §Patients could have both inflammatory disease and advanced disease. ¶Each tumour could have multiple types and characteristics recorded.

Table 1: **Patient and tumour characteristics at baseline**

of six cycles in total, at the discretion of the treating consultant.

Gemcitabine was 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.

Cardiac toxicity was not anticipated at the cumulative doses of epirubicin of 360 mg/m². However, 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.

In the event of gemcitabine-related pulmonary toxicity of CTCAE grade 2 or worse, the patient was discontinued from study therapy.

Paclitaxel infusion was stopped if mild symptoms of skin rash, flushing, and localised pruritus occurred. Intravenous steroids and antihistamines were given and immediate slow rechallenge of chemotherapy was used on recovery. Also, 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. 48 h of steroids were then advised before cautious paclitaxel rechallenge. If severe symptoms occurred, including bronchospasm, generalised urticaria, angio-oedema, hypotension (systolic blood pressure <100 mm Hg), or life-threatening anaphylaxis, paclitaxel infusion was stopped and treatment was given with intramuscular epinephrine 1 mL 1:1000, intravenous steroids, and intravenous antihistamines; rechallenge was contraindicated.

Surgery (breast and axillary), radiotherapy, and adjuvant endocrine treatment were given according to local protocols. Patients with HER2-positive disease did not receive neoadjuvant trastuzumab, but received adjuvant trastuzumab according to local protocols. Clinical surveillance was continued for 5 years at the clinical centres, and after 5 years from the Office for National Statistics (ONS). Patients will continue to receive follow-up through the National Cancer Intelligence Network (NCIN) who will monitor for DFS and overall survival.

Statistical analysis

Our power calculations assumed that the pCR would be 20% after standard treatment (epirubicin and cyclophosphamide followed by paclitaxel). On this basis, we aimed to randomly allocate 200 patients into each of the four treatment groups, yielding combined data for 400 patients into each group for the two questions (ie, efficacy and safety of the addition of gemcitabine to paclitaxel plus epirubicin and cyclophosphamide treatment [component analysis] and order of chemotherapy regimens [sequencing analysis]). This would allow an absolute difference in the pCR in excess of 10% to be detected at the 5% (two-sided) level of significance with 85% power. Statistical analysis was undertaken on an intention-to-treat basis and all protocol violators were analysed within their randomised groups. All reported p values are two-sided.

For the primary analysis, we calculated pCR for all treatment groups and used univariate logistic regression to test the addition of gemcitabine and the role of sequencing. We used multivariate logistic regression to calculate p values for both the treatment and scheduling effects after adjustment for prognostic factors.

We calculated DFS from the date of randomisation to the date of first event (locoregional relapse, distant relapse, progression on neoadjuvant chemotherapy, or death), or to the date of censoring. We calculated overall survival from the date of randomisation to the date of death or to the date of each patient's last clinic visit (for women who are not known to have died). We constructed Kaplan-Meier curves and compared the main treatment effect and main scheduling effect with log-rank tests. We used Cox proportional-hazards models to assess and adjust for prognostic factors. When assessing the association between pathological response and these outcomes, we calculated overall survival and DFS from date of surgery.

The Neo-tAnGo protocol stated that the first planned interim analysis of DFS and overall survival would occur when at least 120 events had occurred or when median follow-up was at least 3 years. We report the results of this first protocol-stated interim analysis of overall survival and DFS.

The methods for dose intensity calculations have previously been described.[18] We compared course-delivered dose intensities (CDDI) across treatment groups with Wilcoxon rank sum tests and Fisher's exact tests.

We compared number of patients with severe toxicity (CTC grade ≥3, or 2 for alopecia or superficial thrombophlebitis) during chemotherapy across treatment groups.

Analyses were done by Warwick Clinical Trials Unit with SAS statistical software (version 9.3).

This study is registered with EudraCT (2004-002356-34), ISRCTN (78234870), and ClinicalTrials.gov (NCT00070278).

Role of the funding source

The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding authors (HME and LH) had full access to all of the data and had final responsibility for the decision to submit for publication.

Results

Between Jan 18, 2005, and Sept 28, 2007, we recruited 831 patients at 57 centres; three patients were found to be ineligible after randomisation, leaving 828 for analysis (figure 1; table 1). Protocol violations occurred in 27 patients according to the trial management committee (figure 1). 13 of these violations were due to

	n	Component analysis			Sequencing analysis		
		EC and paclitaxel (n=404)	EC and paclitaxel-gemcitabine (n=408)	p value*	EC followed by paclitaxel with or without gemcitabine (n=406)	Paclitaxel with or without gemcitabine followed by EC (n=406)	p value*
pCR	..	70 (17%, 14–21)	71 (17%, 14–21)	0·98 (0·95†)	59 (15%, 11–18)	82 (20%, 16–24)	0·03 (0·02†)
HER2-negative	505	14% (10–19)	16% (12–21)	0·46	13% (9–17)	18% (13–23)	0·03
HER2-positive	187	21% (13–30)	22% (14–32)	..	17% (10–26)	26% (17–36)	..
ER-negative	269	32% (24–41)	31% (23–40)	0·97	30% (23–39)	33% (25–42)	0·02
ER-positive	543	10% (7–14)	11% (7–15)	..	7% (4–10)	14% (10–19)	..
Grade 1–2‡	263	7% (4–13)	6% (3–12)	0·19	7% (3–13)	7% (3–12)	0·10
Grade 3‡	324	22% (16–29)	30% (23–38)	..	21% (15–29)	31% (24–38)	..
ER-positive, HER2-negative	348	7% (4–11)	8% (5–14)	0·61	5% (2–9)	10% (6–15)	0·02
ER-positive, HER2-positive	121	17% (9–29)	19% (10–31)	..	9% (4–19)	28% (17–42)	..
ER-negative, HER2-negative	157	32% (21–43)	32% (22–43)	..	29% (19–40)	35% (25–47)	..
ER-negative, HER2-positive	66	26% (13–44)	28% (14–47)	..	32% (17–51)	23% (10–40)	..
ER-positive, HER2-negative (grade 1–2)	147	4% (1–11)	3% (0–10)	0·23	4% (1–11)	3% (0–10)	0·03
ER-positive, HER2-negative (grade 3)	110	12% (5–23)	16% (7–29)	..	7% (1–18)	18% (10–30)	..
ER-positive, HER2-positive (grade 1–2)	38	10% (1–32)	6% (0–27)	..	0% (0–17)	17% (4–41)	..
ER-positive, HER2-positive (grade 3)	55	23% (8–45)	30% (16–49)	..	14% (4–33)	41% (22–61)	..
ER-negative, HER2-positive (grade 1–2)	21	25% (5–57)	11% (0–48)	..	33% (7–70)	8% (0–38)	..
ER-negative, HER2-positive (grade 3)	30	23% (5–54)	41% (18–67)	..	38% (15–65)	29% (8–58)	..
ER-negative, HER2-negative (grade 1–2)	21	0% (0–37)	23% (5–54)	..	10% (0–44)	18% (2–52)	..
ER-negative, HER2-negative (grade 3)	94	31% (18–47)	39% (25–54)	..	30% (17–45)	40% (26–56)	..

Data are n (%, 95% CI) or % (95% CI), unless otherwise indicated. pCR was defined as no invasive disease in breast or axillary lymph nodes. EC=epirubicin-cyclophosphamide. *p value for treatment groups, after adjustment for the factor, unless otherwise stated. †Adjusted for the five stratification variables (age, ER status, tumour size, clinical involvement of axillary nodes, and inflammatory or locally advanced disease). ‡Tumour grade of each patient's largest breast tumour at baseline.

Table 2: **Rates of pathological complete response (pCR)**

omission of the final cycle of chemotherapy, with the patient proceeding straight to surgery.

Because the Neo-tAnGo protocol did not include neoadjuvant trastuzumab, we expected fewer patients with HER2-positive disease to enrol as the trial progressed. 107 patients enrolled in 2005–06 had HER2-positive disease (30% of the 357 tested) compared with 84 in 2007 (24% of the 347 tested; p=0·19).

All patients had surgery (breast and axilla) according to local protocols and the details will form the basis of a future publication. All patients with ER-positive disease (543 [67%] patients were ER-positive) received appropriate adjuvant hormonal treatments and 750 patients (91%) received radiotherapy treatments according to local protocols. 183 (96%) of 191 patients with HER2-positive disease received adjuvant trastuzumab.

812 eligible patients had available pathology reports from surgery after chemotherapy. 141 (17%) of these patients had pCR, which was much the same for patients in the epirubicin and cyclophosphamide plus paclitaxel, and the epirubicin and cyclophosphamide plus paclitaxel and gemcitabine groups (between-group difference 0·07%, 95% CI –5 to 5; table 2). However, significantly more patients who received paclitaxel (with or without gemcitabine) before epirubicin and cyclophosphamide achieved pCR than did those who received epirubicin and cyclophosphamide first (between group difference 6%, 0·5 to 11; table 2). In post-hoc analysis, we noted no interaction between component and sequence (p=0·86), suggesting the effects were independent from each other. Adjustment by stratification factors did not affect the results (table 2). Subgroup analysis by HER2 status, ER status, or tumour grade did not affect the results (table 2, appendix).

See Online for appendix

Median follow-up was 47 months (IQR 37–51). At the time of analysis, 167 (20%) of the 828 eligible patients had died, 227 (27%) had had locoregional or distant relapses, and 236 (29%) had had DFS events. We noted no significant difference in DFS or overall survival between treatment components or treatment sequences (figure 2). Adjustment for stratification variables did not affect these findings (figure 2).

Full treatment details were available for 819 (99%) of 828 patients (410 patients in the epirubicin and

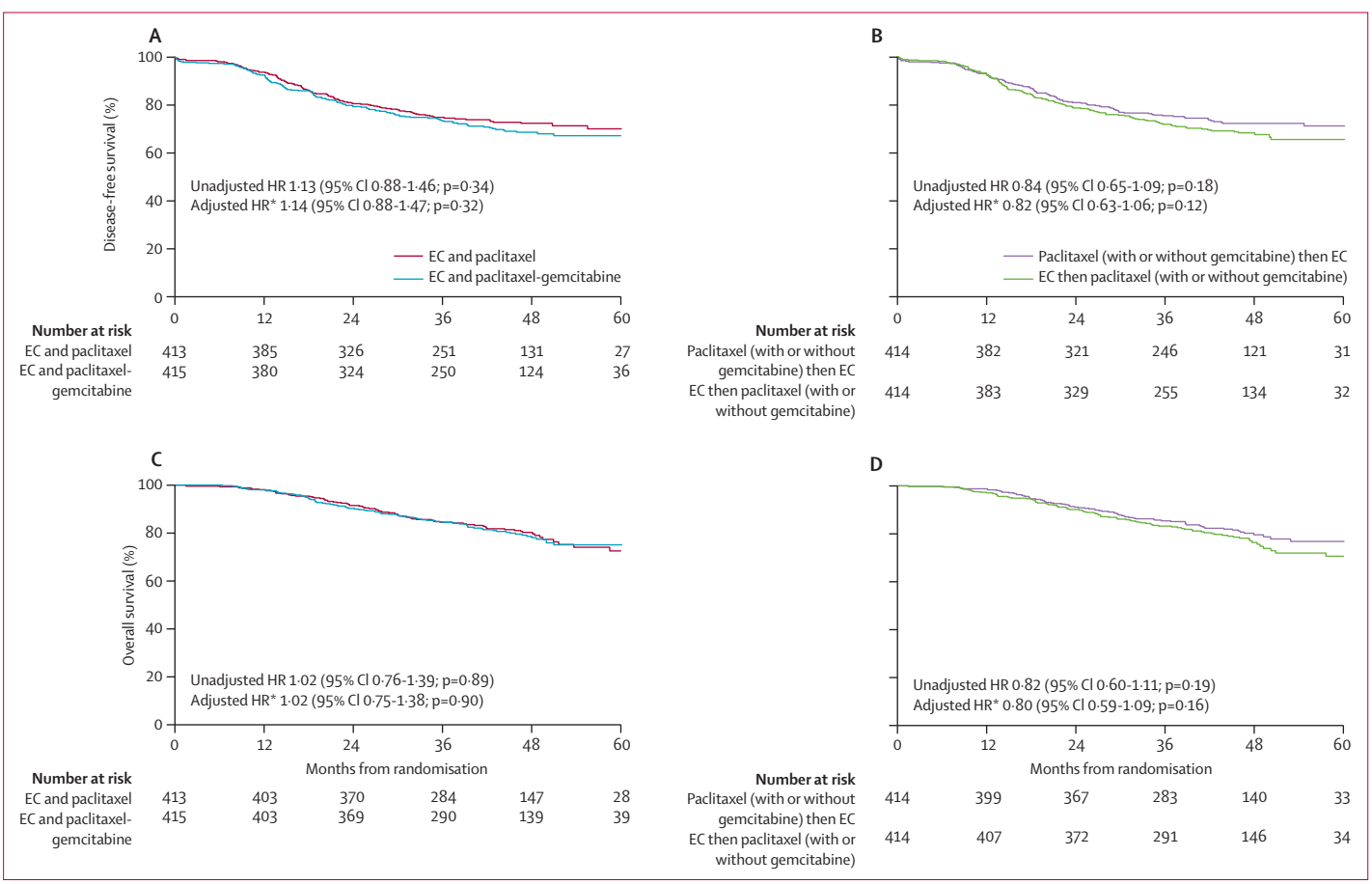

Figure 2: **Disease-free survival and overall survival from randomisation**
(A) Disease-free survival by treatment component. (B) Disease-free survival by treatment sequence. (C) Overall survival by treatment component. (D) Overall survival by treatment sequence
EC=epirubicin and cyclophosphamide. HR=hazard ratio. *Adjusted for stratification variables.

cyclophosphamide and paclitaxel group *vs* 409 patients in the epirubicin and cyclophosphamide, then paclitaxel and gemcitabine group; 413 patients in the epirubicin and cyclophosphamide followed by paclitaxel with or without gemcitabine group *vs* 406 patients in the paclitaxel with or without gemcitabine followed by epirubicin and cyclophosphamide group). Seven patients received no treatment cycles at all, mainly because of patient choice or early relapse. 702 (86%) of the remaining 812 patients received eight cycles of chemotherapy; the main reasons for not receiving all eight cycles were toxicity (54 [49%] of the 110 patients receiving between one and seven cycles), disease progression (28 [25%] patients), allergic reaction to paclitaxel (15 [14%] patients), poor response to chemotherapy (seven [6%] patients), or other reasons (six [5%] patients).

The median CDDI for the 819 patients was 97% (IQR 91–99%). We noted no differences in CDDI between the component groups or the sequencing groups (p=0·96 for component analysis and p=0·46 for sequencing analysis; appendix). 84% of patients received optimum CDDI (≥85%). No differences between the component groups or the sequencing groups were detected (p=0·56 for component analysis and p=0·18 for sequencing analysis).

Dose intensities over individual cycles did not notably deteriorate for any of the four randomised treatment groups (appendix). Moreover, no differences of note existed between either the taxane-containing and anthracycline-containing regimens, or between the four randomised treatment groups. More dose reductions for the fourth cycle of regimens containing paclitaxel (with or without gemcitabine) occurred when it was given second compared with prior administration (22% *vs* 10%, respectively; appendix), and similarly for dose delays (15% *vs* 11%, respectively; appendix).

Overall survival from surgery was longer for patients attaining pCR than it was for those who did not attain pCR (figure 3). 12 (9%) of 141 patients with pCR died, as did 147 (22%) of 671 patients without a pCR. In our analysis of DFS, 18 (13%) of 141 patients with pCR relapsed or died compared with 206 (31%) of 671 patients who did not achieve a pCR (figure 3).

GCSF use was reported in 515 (8%) of 6189 cycles (appendix); 270 (52%) within taxane-containing cycles. 179 (22%) of 812 patients received GCSF, which was similar across treatment groups (p=0·90 for the component analysis and p=0·51 for the sequencing analysis; appendix). Incidence of severe toxicities (grade 3–4) by cycle and patient are shown in table 3 and table 4 and mild toxicities (grade 1–2) are reported in the appendix. Adverse events were as expected. Grade 3 toxicities included neutropenia, muscle and joint pain, infection, neuropathy, transaminitis, fatigue, nausea and vomiting, rash, and acute hypersensitivity (table 3). Grade 4 toxicities included neutropenia and infection (table 4). Accelerated paclitaxel (with or without gemcitabine) was both tolerable and deliverable.

Only two patients died during chemotherapy. One patient in the paclitaxel followed by epirubicin and cyclophosphamide group died of neutropenic sepsis and pneumonia 11 days after their third dose of paclitaxel. One patient in the epirubicin and cyclophosphamide followed by paclitaxel group died of venous thromboembolism 2 days after the third dose of epirubicin and cyclophosphamide. Another two patients died within 3 months of completion of chemotherapy but causes were unrelated to treatment. One patient in the epirubicin and cyclophosphamide followed by paclitaxel group received only five cycles of chemotherapy because of fatigue and anaphylaxis and then died 12 weeks later of respiratory failure (distant recurrence with bilateral pleural effusion and extensive alveolar shadowing). One patient in the paclitaxel and gemcitabine followed by epirubicin and cyclophosphamide group completed only seven cycles of chemotherapy because of disease progression and died 5 weeks later from recurrence of leptomeningeal breast cancer.

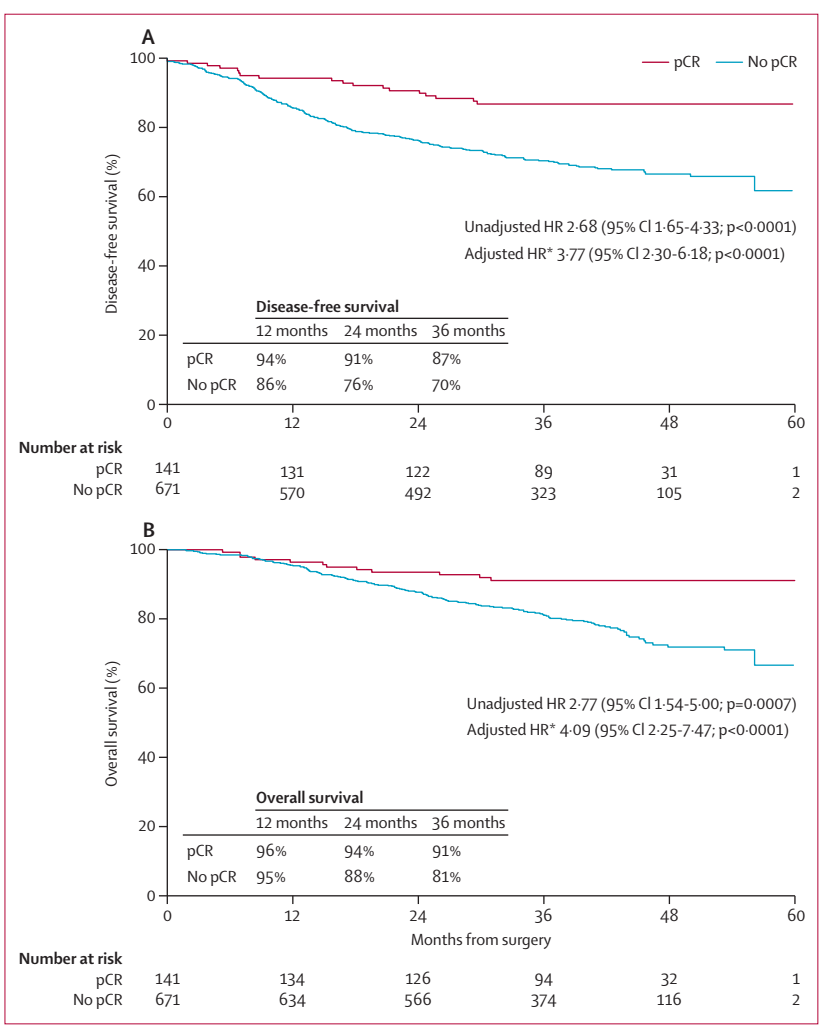

Figure 3: **Disease-free survival and overall survival from surgery by pathological complete response**
(A) Disease-free survival. (B) Overall survival. pCR=pathological complete response. HR=hazard ratio. *Adjusted for stratification variables.

	EC followed by paclitaxel				Paclitaxel followed by EC				EC followed by paclitaxel-gemcitabine				Paclitaxel-gemcitabine followed by EC			
	EC		Paclitaxel		Paclitaxel		EC		EC		Paclitaxel-gemcitabine		Paclitaxel-gemcitabine		EC	
	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients
Neutropenia	35	28	28	21	15	13	48	36	40	29	27	22	11	10	42	31
Infection	15	14	4	3	4	3	11	10	12	11	7	7	9	8	14	13
Fatigue	8	6	6	4	8	5	5	4	2	2	7	7	11	9	10	8
Muscle or joint pain	..	..	10	10	10	8	..	..	..	..	19	15	10	6	4	3
Vomiting	13	11	1	1	..	..	10	7	10	9	..	..	..	..	11	9
Nausea	7	6	1	1	..	..	14	10	14	12	1	1	..	..	7	7
Neurosensory	..	..	11	10	7	7	1	1	..	..	10	9	8	7	2	2
Transaminitis	..	..	..	..	2	2	1	1	1	1	17	9	15	10	1	1
Rash	..	..	3	3	3	3	..	..	..	..	1	1	14	13	..	..
Fever	6	5	1	1	..	..	2	2	2	2	..	..	2	2	5	5
Acute hypersensitivity	..	..	2	2	1	1	..	..	..	..	3	3	10	10	..	..
Diarrhoea	..	..	..	..	..	..	1	1	1	1	2	2	3	3	4	4
Constipation	..	..	..	..	5	2	..	..	1	1	1	1	3	2	..	..
Dyspneoa	1	1	1	1	..	..	..	..	..	..	..	..	2	2	2	2
Anaemia	2	2	1	1	..	..	..	..	..	..	..	..	..	..	..	..
Thrombocytopenia	..	..	..	..	..	..	3	2	..	..	..	..	..	..	..	..
Cough	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2	2
Deep vein thrombosis	..	..	..	..	..	..	..	..	1	1	..	..	1	1	..	..
Stomatitis	..	..	..	..	1	1	..	..	..	..	..	..	..	..	..	..

Alopecia and superficial thrombophlebitis do not have a common terminology criteria for adverse events (CTCAE) grade of 3. EC=epirubicin-cyclophosphamide. ..=no reported toxic effects of this grade.

Table 3: **Reported grade 3 severe toxic effects**

	EC followed by paclitaxel				Paclitaxel followed by EC				EC followed by paclitaxel-gemcitabine				Paclitaxel-gemcitabine followed by EC			
	EC		Paclitaxel		Paclitaxel		EC		EC		Paclitaxel-gemcitabine		Paclitaxel-gemcitabine		EC	
	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients	Cycles	Patients
Neutropenia	17	14	3	3	7	5	17	15	31	21	8	7	7	7	25	19
Infection	..	..	1	1	1	1	..	..	..	..	..	..	..	..	1	1
Fatigue	2	1	..	..	..	..	..	..	..	..	..	..	1	1	1	1
Muscle or joint pain	..	..	1	1	..	..	..	..	..	..	..	..	..	..	..	..
Vomiting	..	..	..	..	..	..	..	..	3	2	..	..	1	1	..	..
Neurosensory	1	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..
Rash	..	..	..	..	..	..	..	..	..	..	1	1	..	..	..	..
Diarrhoea	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..
Constipation	..	..	..	..	..	..	..	..	..	..	..	..	1	1	..	..
Dyspnoea	..	..	..	..	..	..	1	1	..	..	..	..	..	..	..	..

Alopecia, superficial thrombophlebitis, and cough do not have a common terminology criteria for adverse events grade of 4. EC=epirubicin-cyclophosphamide. ..=no reported toxic effects of this grade.

Table 4: **Reported grade 4 severe toxic effects**

Discussion

In our study, provision of a taxane before standard anthracycline chemotherapy was associated with a significant improvement in pCR, from 15% to 20%, compared with a standard anthracycline-first sequence (p=0·03; panel). Addition of gemcitabine did not provide significant benefit overall. Achievement of a pCR was correlated with significant improvement in DFS (p<0·0001) and overall survival (p=0·0007). These findings confirm the benefit of taxane-first sequencing that was noted in a large retrospective non-randomised study from the MD Anderson Cancer Center, TX, USA,[19] which reported data from 1414 patients treated with neoadjuvant chemotherapy and 1596 patients treated with adjuvant chemotherapy between 1994 and 2009. 88% of these patients received paclitaxel-first sequencing and showed increased pCR (20·9% *vs* 12·4%), and fewer relapses occurred in the neoadjuvant patient group.

Panel: **Research in context**

Systematic review

We searched PubMed for listed articles on neoadjuvant chemotherapy from 1980 onwards. In addition all major international early breast cancer trials groups were contacted to discuss our proposed design of epirubicin and cyclophosphamide, and dose-dense paclitaxel with or without gemcitabine. The NSABP-B38 adjuvant trial had a similar experimental arm with dose-dense doxorubicin and cyclophosphamide, and dose-dense paclitaxel (175 mg/m² every 2 weeks) with or without gemcitabine (2000 mg/m² every 2 weeks). This trial was published in 2013,[14] and showed no benefit to the addition of gemcitabine. The NSABP-B40 neoadjuvant trials added gemcitabine or capecitabine and at publication[15] in 2012 showed no benefit. Neo-tAnGo was unique in also addressing the question of paclitaxel-first versus standard anthracycline-first sequencing. In addition, Neo-tAnGo had a complementary adjuvant trial (tAnGo) which also addressed the addition of gemcitabine.

Interpretation

Neo-tAnGo confirms the NSABP-B38,[14] NSABP-B40,[15] and tAnGo[11] results, which show no benefit from the addition of gemcitabine to anthracycline and taxane-based chemotherapy treatments for early breast cancer. Neo-tAnGo shows a significant benefit from taxane-first sequencing, and is the largest randomised trial to confirm this effect.

314 patients have been included in small randomised phase 2 adjuvant trials[20–24] and 276 patients have been included in neoadjuvant trials[25–28] addressing taxane and anthracycline sequencing. Some of these studies report more dose reductions of taxane when anthracyclines are given first,[21–23,25] and one randomised neoadjuvant study showed a non-significant increase in incidence of pCR for taxane-first sequencing.[27] Indirect evidence from neo-adjuvant randomised studies for taxane-first sequencing comes from EORTC 10994,[28] which compared fluorouracil, epirubicin, and cyclophosphamide with docetaxel followed by epirubicin and docetaxel. The HR for DFS benefit was 0·85 (stratified log-rank p=0·035). Neo-tAnGo confirms the improvement in pCR with taxane-first sequencing suggested by these previous studies. However, the Neo-tAnGo analysis showed no sequence-specific difference in CDDI to explain this taxane-first sequence result.

Preclinical and clinical studies have suggested mechanisms to explain the benefit of early receipt of taxanes. Cross-resistance has been shown to emerge dependent on sequence. An in-vitro study of MCF-7 cells[29] showed that when these cells were made resistant to doxorubicin, cross-resistance to paclitaxel increased 4700 times. However when MCF7 cells were made resistant to paclitaxel, the cross-resistance for doxorubicin was only four times higher. Pachmann and colleagues[30] describe release of breast cancer circulating tumour cells (CTCs) during paclitaxel treatment and a decrease in CTCs during anthracyclines. The study[30] suggests that increased long-term benefit might be achieved by taxane-first sequencing. Taghian and colleagues[26] drew attention to increases in interstitial fluid pressure and improved oxygenation within breast tumours after neoadjuvant paclitaxel, which might allow increased concentrations of anthracyclines to accumulate within tumour tissue when given afterwards. In addition, preclinical data show that in breast cancer cells with heat shock protein 27 overexpression, a paclitaxel then doxorubicin sequence is more effective at cell killing than the more standard doxorubicin then paclitaxel sequence.[31]

Taxane-first sequences allow early treatment with concomitant trastuzumab and bevacizumab, which might provide additional therapeutic advantage, shown for trastuzumab in the FinHer study.[32] Both the NSABP-B40[15] and ARTemis[33] trials of bevacizumab have adopted taxane-first sequencing for this reason. Because all patients receive both the taxane and anthracycline components, taxane-first sequencing could be considered in all similar block-sequential adjuvant and neoadjuvant protocols.

Neo-tAnGo confirms the result of tAnGo[11] and shows no significant benefit from addition of gemcitabine to a combination that already contains anthracycline, cyclophosphamide, and paclitaxel. However, gemcitabine has shown promising results in patients with metastatic disease when added to paclitaxel in the first-line relapse setting.[13] Much the same results have already been reported in the adjuvant NSABP-B38,[14] and neoadjuvant NSABP-B40[15] studies. NSABP-B38 showed no benefit for gemcitabine with paclitaxel used in the same dose and schedule as in NeotAnGo. NSABP-B40 had a complex design, but for the purposes of discussion here, addressed the addition of gemcitabine to docetaxel. Gemcitabine was given at 666 mg/m² per week (compared with 1000 mg/m² per week in NeotAnGo and NSABP-B38) and docetaxel doses were reduced to 75 mg/m² , and showed no benefit in terms of pCR. In Neo-tAnGo, we noted a non-significant increase in pCR after the addition of gemcitabine in patients with high-grade disease (table 2).

Paclitaxel was delivered every 14 days at 175 mg/m² (with or without gemcitabine 2000 mg/m²). This dose-dense protocol was delivered without any reported delays in 82% of paclitaxel-containing cycles and only 8% of cycles required growth factor support to maintain dose intensity. This schedule allowed a 50% increase in dose density compared with dosing once every 21 days and also reduced the total time of chemotherapy. This strategy provides a cost-effective and well-tolerated way to achieve dose intensification of paclitaxel. The use of neoadjuvant or adjuvant weekly paclitaxel is now often used as standard at 80 mg/m² per week and therefore the taxane-first sequencing benefit seen in Neo-tAnGo with paclitaxel at 87·5 mg/m² per week, is close to standard practice across the world.

Whether pCR in neoadjuvant trials can be a surrogate endpoint for DFS and overall survival has been debated. von Minckwitz and colleagues' meta-analysis[34] of 6377 patients in the German Breast Group and ABOBSG neoadjuvant chemotherapy trials showed that the prognostic value of not achieving a pCR is most dependent on the molecular subtype of the original

tumour. Patients with luminal A tumours and a lower pCR nevertheless have a better prognosis than do other subgroups in terms of DFS and overall survival. By comparison with overall pCR reported in the von Minckwitz meta-analysis,[34] pCR in Neo-tAnGo was lower in all groups. For luminal A subtype,[35] a meta-analysis included 46% of patients in this category with pCR of 8·9%, whereas Neo-tAnGo had 29% luminal A patients with pCR of 3·5%. In addition, 662 (50%) of 1327 patients with HER2-positive disease in the meta-analysis received neoadjuvant trastuzumab, whereas none of the 187 patients with HER2-positive disease in Neo-tAnGo did. Neoadjuvant trastuzumab increased the pCR by an absolute of 15% in these 662 patients in the meta-analysis. However in the triple-negative subgroup, pCR rates are much the same between studies. The meta-analysis shows pCR of 35·8% in 911 patients, although in a recent trial (GEPAR Quinto),[36] 663 patients with triple-negative breast cancer had a pCR of 27·9%, increasing to 39·3% after the addition of bevacizumab to chemotherapy. The pCR for 94 such patients in Neo-tAnGo was 39% with the addition of gemcitabine and 40% with taxane-first sequence.

In the Z1040-Alliance neoadjuvant study,[37] 280 patients with HER2-positive breast cancer were randomly allocated to receive standard fluorouracil, epirubicin, and cyclophosphamide followed by weekly paclitaxel with trastuzumab (pCR in the breast 56·5%), and a taxane-first sequence with concurrent weekly trastuzumab throughout (pCR in the breast 54·2%). However in Neo-tAnGo, 187 patients with HER2-positive disease had a pCR for taxane-first sequence of 26% compared with 17% for standard sequence (p=0·03; table 2). Notably, in Neo-tAnGo, in 121 ER-positive, HER2-positive women, pCR was 28% for taxane-first sequencing and 9% for anthracycline-first sequencing, and in 66 ER-negative, HER2-positive women pCR was 23% for taxane-first sequencing and 32% for anthracycline-first sequencing. The Neo-tAnGo subgroups are too small to draw conclusions and the results can only be hypothesis-generating. However, the differences between the Neo-tAnGo results and Z1040 could be explained by the absence of neoadjuvant trastuzumab in Neo-tAnGo.

A recent meta-analysis[38] by the US Food and Drug Administration (FDA) confirms the strong correlation between pCR and DFS and overall survival in patients achieving a pCR, and this association was confirmed in Neo-tAnGo. However, the association is complex. The FDA proposed that pCR in neoadjuvant trials in highly proliferative breast cancers could now contribute to accelerated drug approval in these types of early breast cancer.[39] This proposal is a landmark in neoadjuvant chemotherapy trials for highly proliferative breast cancer, and will facilitate more rapid introduction of new agents for this subtype. Randomised neoadjuvant trials of chemotherapy in combination with target-directed novel agents will be able to focus on high proliferation molecular subtypes (ER-negative, HER2-positive, high grade, and patients with germline *BRCA* mutations) in which the association between pCR and DFS and overall survival is expected to be correlated.

However, the FDA meta-analysis also indicates subgroups of breast cancer with lower proliferative potential (ie, ER-positive, HER2-negative, low and intermediate grade) that have a better prognosis independent of pCR. In these groups, the response to chemotherapy is poor and pCR is low but the long-term outcome is good, and survival is influenced more by subsequent adjuvant hormonal treatment. In Neo-tAnGo, patients from all the different prognostic groups were included, and therefore the profile of the trial population will weaken the correlation between pCR and long-term outcomes for the group as a whole. In future, neoadjuvant clinical trials will probably include specific prognostic groups. For patients with a previously known poor prognosis, who have high pCR incidence, a robust and reliable correlation will probably emerge between pCR and longer-term outcome.

Contributors

HME, A-LV, LH, GW, JD, and CC designed the study and wrote grant applications. HME, A-LV, LH, NF, JY, and JD coordinated the trial and data collection. LH analysed the data and wrote the report. HME, A-LV, LH, MI, JA, LH-D, IG, CG, JD, EP, and CC interpreted the data and contributed to writing of the report. HME, MI, JA, LH-D, IG, KM, SH, TH, AS, SC, SD, DR, RL, MH, CG, and GW recruited patients. All authors collected data, revised the report and agreed to submit the paper for publication.

Conflicts of interest

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