STUDY PROTOCOL

Muti-center trial

National Taiwan University Hospital

Taipei Veterans General Hospital

Chang Gung Memorial Hospital

Tri-Service General Hospital

Mackay Memorial Hospital

Taichung Veterans General Hospital

China Medical University Hospital

Changhua Christian Hospital

Kaohsiung Veterans General Hospital

Kaohsiung Medical University Chung-Ho Memorial Hospital

Protocol No:

A Randomized Phase III Study of Docetaxel/ Epirubicin versus Tailored Regimens as Neoadjuvant Chemotherapy for Stage II/III Breast Cancer with Tumor Size More Than 3 cm

The information contained in this document is privileged and confidential and, except to the extent necessary to obtain Ethics Committee approval and informed consent may not be disclosed to a third party.

Amendments

No.	Date
1	20-Oct-08
2	28-Oct-08
3	

CONTENTS

List of Abbreviations and Definitions of Terms

Abbreviation	_Definition_
AE	Adverse event
ALT	Alanine aminotransferase
ALP	Alkaline phosphatase
ANC	Absolute neutrophil count
AST	Aspartate transaminase
Bil(T)	Total bilirubin
CBC	Complete blood count
CR	Complete response
CRu	Complete response/unconfirmed
CRF	Case report form
CT	Computed tomography
CV	Curriculum vitae
D/C	Differential count
DCR	Disease control rate
DOH	Department of Health
ECG	Electrocardiogram
ECOG	Eastern Cooperative Oncology Group
GCP	Good clinical practice
IHC	Immunohistochemical stain
IRB	Institutional Review Board
MRI	Magnetic resonance imaging
NCI-CTC	National Cancer Institute common toxicity criteria
ORR	Objective response rate
OS	Overall survival
PD	Progressive disease

PFS	Progression-free survival
PR	Partial response
RECIST	Response evaluation criteria in solid tumor
SAE	Serious adverse event
SD	Stable disease
SDV	Source data verification
SPD	Sum of the products of the greatest diameter
WBC	White blood cell
WOCBP	Women of childbearing potential
TE	Docetaxel plus epirubicin regimen
Topo II	Topoisomerase II
TS	Thymidine synthase
UNL	Upper normal limit

Synopsis

Title of Study:	A Randomized Phase III Study of Docetaxel/ Epirubicin regimen versus tailored regimens as Neoadjuvant Treatment for Stage II/III Breast Cancer with Tumor Size More Than 3 cm
Objectives:	**Primary objective** To evaluate and compare the pathological complete response (pCR) rates after neoadjuvant chemotherapy with tailored chemotherapeutic regimens or TE for stage II/III breast cancer with tumor size more than 3 cm **Secondary objectives** 1. To evaluate the overall clinical response rate 2. To evaluate the safety profiles
Study Design:	Open-label, multicenter randomized phase III study. Eligible patients will be randomized in 1:1 ratio into 2 arms: Arm A: Control regimen: docetaxel-epirubicin for 4 cycles before surgery. Arm B: Tailored regimens, base on immunohistochemical study of the tumor biopsy tissue, for 4 cycles before surgery.
Sample Size:	Under the assumption of pCR rate of 15% in TE arm, to achieve 80% power at the 5% level of significance for the detection of a 15% increase of pCR rate in tailored regimen arm, 136 patients in either arm should be included in the study. If a 10% drop-out rate and 5% multi-center study variation effect are considered, a total of 316 patients will be required.
Criteria for Inclusion and Exclusion:	Inclusion criteria To be eligible for inclusion, each patient must fulfill all of the following criteria: 1. Histologically confirmed invasive, but non-inflammatory, breast carcinoma, with stage II or III disease (AJCC 7th ed) 2. And, tumor size more than 3 cm in greatest diameter measured by estimated by CT scan or MRI 3. Documented Her2/neu negative , including score 0, 1+, or 2+ by immunohistochemistry 4. No prior radiotherapy, hormonal therapy or chemotherapy for invasive breast cancer 5. Performance status of ECOG 0, 1, 6. Female with age older than 20 years

	7. Laboratory parameter
	
	    A. Absolute neutrophil count (ANC) $\geqq$ 1500/mm3
	
	    B. Total bilirubin $\leqq$ 2.0 times the upper limit of normal (ULM)
	
	    C. AST or ALT $\leqq$ 2.5 times the upper limit of normal (ULM)
	
	    D. Platelets $\geqq$ 100,000/mm3
	
	    E. Serum creatinine $\leqq$ 1.5 x ULM
	
	    F. Fasting triglyceride $\geqq$ 70 mg/dL
	
	8. Ability to understand and willingness to sign a written informed consent document.
	
	Exclusion criteria
	
	Patients who fulfill any of the following criteria will be excluded from the trial:
	
	1. Evidence of metastatic breast cancer or inflammatory breast cancer
	
	2. Bilateral breast cancer, metaplastic carcinoma, or mucinous carcinoma
	
	3. Known allergy to any of the study drugs or to agents containing Cremophor.
	
	4. Serious intercurrent infections or medical illnesses that are uncontrolled or the control of which may be jeopardized by this therapy
	
	5. Psychiatric disorders or other conditions regarding the subject incapable of complying with the requirements of the protocol
	
	6. Evidence of baseline sensory or motor neuropathy
	
	7. Pregnant or breast feeding women
	
	8. Previous or current systemic malignancy with the exception of curatively treated non-melanoma skin cancer or cervical carcinoma in situ with a disease-free interval of at least 5 years

Study Medication:	Control group: Tax 35 mg/m^2 1hr infusion / Epi 45 mg/m^2 (TE) iv infusion. day 1 and day 8 Tailored chemotherapy group: 7 tailored chemotherapy regimens according to the expression of 3 biological predictor markers from biopsy tumor samples. Tau+ topo II+ ERCC1+ : Epi 45mg/m^2 iv / 5FU 2000mg/m^2 + Lv 300 mg/m^2 24 hrs infusion, day 1 and day 8 Tau+ topo II+ ERCC1- : Epi 45mg/m^2 iv/ Cis 35mg/m^2 24 hrs iv infusion day 1 and day 8. Tau+ topo II- ERCC1+ : Vin 25mg/m^2 iv / 5FU 2000mg/m^2 + Lv 300 mg/m^2 24 hrs infusion, day 1 and day 8 Tau+ topo II- ERCC1- : Cis 35mg/m^2 24 hrs infusion / Vin 25mg/m^2 iv day 1 and day 8. Tau- topo II+ ERCC1+ : TE

	Tau- topo II+ ERCC1- : TE Tau- topo II- ERCC1+: Tax 35mg/m^2 1hr infusion /, 5FU 2000mg/m^2 + Lv 300 mg/m^2 24 hrs infusion, day 1and day 8 Tau- topo II- ERCC1- : Tax 35mg/m^2 1hr infusion / Cis 35mg/m^2 24 hrs infusion day 1 and day 8 Each regimen will be given on a 3 weeks cycle for up to 4 cycles, with GCSF support. Abbre: Tax: docetaxel, Epi: epirubicin, Cis: cisplatin, Vin: vinorelbine, Lv: leucovorin, 5FU: 5-fluorouracil
Therapeutic Assessment:	1. Pathological response rate 2. Clinical response rate 3. Safety
Planned biomarkers analyses for determining the tailored regimen	Topo II α (Topoisomerase II), ERCC1(Excision repair cross-complementing genes), Tau
Planned other biomarkers analysis for retrospective analysis	Other docetaxel, epirubicin, cisplatin, vinorelbine, 5FU associated drug resistance/sensitivity predicting marker, such as c-myc, P53, thymidylate synthase (TS) etc., and cDNA (complementary Deoxyribonucleotide) micro-array study.

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Schema

Stage II/III breast cancer with size > 3 cm
(HER-2/neu negative)

Randomization: 1:1
Arm A: TE
Arm B: Tailored chemotherapy (regimens decided by, topo II, tau, and ERCC1 expression status) **(See sec 1.7)**

Neoadjuvant chemotherapy with assigned regimens for 4 cycles

- If tumor size increases by physical examination after any cycle of chemotherapy, image study to evaluate response. If tumor size dose not decrease by physical examination after 2 cycle of chemotherapy, image study to evaluate response.If CR, PR, or SD with tumor size decrease,documented by image study, continue protocol treatment. If PD or SD with tumor size increase more than 1% documented, off protocol treatment. goes to **options**:

Options:
- **Salvage neoadjuvant chemotherapy** with regimens at investigator's discretion.
- or **Surgery,**
- or **Other salvage treatment**

Data of topo II, tau, and ERCC1 expression in arm A patient will be released.

Tumor resection or biopsy

- After tumor resection (either mastectomy or lumpectomy)**,** adjuvant chemotherapy will be given at investigator's discretion.
- After biopsy proves the existence of residual invasive cancer, further neoadjuvant chemotherapy will be given at investigator's discretion.

Data of topo II, tau, and ERCC1 expression in arm A patient will be released.

After complete above treatment,
+ adjuvant radiotherapy if indicated,
± hormone therapy

1. INTRODUCTION

1.1 Breast cancer

Breast cancer is the most common malignancy occurring in women around the world and in Taiwan.[1] The unique features of breast cancer in Taiwan including rather younger onset and more advanced stages of disease at presentation compared to the western countries.[2]

For many decades, surgery remains the major part of the multidisciplinary treatment. Due to a tendency to more conservative surgery recently, the adjuvant chemotherapy has become more validated via meta-analysis of the worldwide experience. It is believed that many women with early-stage breast cancer have micro-metastases at initial diagnosis. Adjuvant chemotherapy for early-stage disease improved disease-free survival and overall survival, presumably by eradicating micrometastatic disease.[3]

1.2 Neoadjuvant chemotherapy for breast cancer

Neoadjuvant chemotherapy describes chemotherapy given before definitive locoregional treatment. Early studies considered the role of neoadjuvant chemotherapy in locally advanced inoperable disease with the view to downstaging tumors to an operable stage. Despite insufficient data from prospective randomized trials of neoadjuvant chemotherapy has become widely accepted as the treatment of choice for patients with locally advanced disease.[4]

For primary operable breast cancer, neoadjuvant chemotherapy is a standard option. A large randomized study (NSABP B-18) comparing neoadjuvant chemotherapy with adjuvant chemotherapy demonstrated identical survival rates in both patients groups and higher breast conservation rate in neoadjuvant groups. Furthermore, pathological complete response (pCR) was associated with significantly improved long-term disease free and overall survival.[5,6] This finding has been validated in many other studies, and pCR rate is now considered to be the most important study endpoint to assess the efficacy of neoadjuvant chemotherapy regimens.

1.3 Rationale of TE regimen

Anthracycline/ taxane-based chemotherapy regimens have been studied extensively in prospective randomized trials and are the most frequently prescribed

treatments in patients with operable breast cancer (Table 1). Regimens that have been tested in large multicenter phase III trials and yielded pCR rates of at least 15% and up to 20% are AC (doxorubicin/ cyclophosphamide) followed by D (docetaxel); DAC (Docetaxel/ doxorubicin/ cyclophosphamide); CVAP (cyclophosphamide/ vincristine/ doxorubicin/ prednisone)   followed by D (docetaxel); and a dose-dense sequence of   E (epirubicin) and T (paclitaxel).

Table 1. Randomized Clinical Trials Exploring Preoperative Chemotherapy				
Author/Trial	No. of Patients	Regimen 1	Regimen 2	pCR (%)
Bear et al, 2003 (NSABP-B27)	2411	AC	AC-D	9.6 v 18.9*
von Minckwitz et al, 2005 (Geparduo)	913	AD	AC-D	11 v 22.3*
Untch et al, 2002	475	ET	dd-ET	10 *v* 18§
Evans et al, 2005	363	AC	AD	16 *v* 12‡
von Minckwitz et al, 2004 (Gepartrio)	286	DAC-DAC	DAC-NX	NR : 7.3 v 3.1 R : 22.9§
von Minckwitz et al, 2001 (Gepardo)	248	AD	AD+Tam	10.3 v 9.1*
Dieras et al, 2004	200	AC	AT	10 *v* 16‡
Steger et al, 2004	292	ED x 3	ED x 6	7.7 *v* 18.6§
Green et al, 2005	258	T (Q3W)	T (QW)	13.7 *v* 28§
Buzdar et al, 1999	174	FAC	T	16.4 *v* 8.1§
Smith et al, 2002 (Aberdeen)	104	CVAP	CVAP-D	15.4 *v* 30.8†
Abbreviations: NSABP, National Surgical Adjuvant Breast and Bowel Project; AC, doxorubicin and cyclophosphamide; AC-D, AC and docetaxel; AD, doxorubicin and docetaxel; AT, doxorubicin and paclitaxel; ET, epirubicin and paclitaxel; dd-ET, dose dense epirubicin and paclitaxel; DAC, docetaxel, doxorubicin, and cyclophosphamide; NX, vinorelbine and capecitabine; Tam, tamoxifen; FAC, fluorouracil, doxorubicin, and cyclophosphamide; CAVP-D, cyclophosphamide, doxorubicin, vincristine, prednisone, and docetaxel; w, weekly; NR, nonresponder; R, responder after two cycles *Breast only, ypT0 regardless of nodal status.				

†Breast only, ypT0/ypTis regardless of nodal status.

‡ypT0, ypN0 only.

§ypT0/ypTis, ypN0.

Currently, regimen containing docetaxel plus anthracycline (either doxorubicin or epirubicin) is regarded as regimen with highest intensity. However, its adverse effects (mainly febrile neutropenia) make this regimen suitable only for patients with good performance status. In this study, we selected epirubicin in order to reduce cumulative cardiotoxicity. Prophylactic G-CSF (granocyte-colony stimulating factor) will be given to reduce the incidence of febrile neutropenia.

1.4 Rationale of tailored therapy for breast cancer

Determining which patients to treat with chemotherapy and choosing optimal treatment would allow practitioners to maximize the benefit of chemotherapy. A predictive marker is defined as a factor that indicates sensitivity or resistance to a specific treatment and may be of value for the tailored therapy. A well-known example is the estrogen receptor in breast cancer, which is widely used to make a decision on hormonal treatment. Several predictive markers have been identified and include oncogenes, tumor suppressor genes, genes involved in angiogenic and apoptotic pathways and cell proliferation and repair and those encoding targets of chemotherapy[7].

Specifically for chemotherapy, the markers encoding targets of chemotherapy and DNA (Deoxyribonucleotide)repair have shown to be most effective in predict the efficacy for specific chemotherapy. The well-known targets of chemotherapy include topoisomerase II (Topo II)[8], tau[9], and thymidine synthase (TS), which have shown to predict the response of anthracycline, taxanes and 5-FU, respectively. The well-known DNA repair related markers include O(6)-methylguanine DNA methyltransferase (MGMT)[10] and excision repair cross-complementing genes (ERCC1)[11], which have shown to predict the response of temozolomide and cisplatin, respectively.

Although tailored therapy has been thought to be promising and there are many identified predictive markers, the efficacy of tailored therapy has never been validated in well designed prospective randomized trial in breast cancer. Because breast cancer is in general sensitive to many chemotherapy agents with identified predictive markers, it is suitable to test the efficacy of tailed therapy. For breast cancer,

neoadjuvant chemotherapy provides an excellent opportunity for objective assessment of treatment-induced tumor response and for studying biomarkers characteristic of therapy-induced tumor responses.

1.5 Rationale of selected biomarkers and specific regimens

Breast cancer is one of the most chemotherapy-sensitive solid tumors. Several classes of cytotoxic agents are active, both single and as components of multidrug regimens. Recommended doses and schedules for commonly used agents are listed in Table 2.

Table 2. Commonly used doses and schedules of selected single chemotherapeutic agents for breast cancer		
Agent	Usual Doses	Usual Route
Capecitabine	1000-2000 mg/m^2 twice daily	PO
Cyclophosphamide	400-600 mg/m^2	IV
	100 mg/m^2 (max 150 mg)	PO
Docetaxel	80-100 mg/m^2	IV
	30-35 mg/m^2	IV
Doxorubicin	40-75 mg/m^2	IV
Liposomal doxorubicin	30-40 mg/m^2	IV
Epirubicin	60-90 mg/m^2	IV
Etoposide	50-100 mg	PO
5-Fluorouracil	400-600 mg/m^2	IV
	500 mg/m^2	Continuous IVF
Gemcitabine	800-1000 mg/m^2	IV
Methotrexate	40-60 mg/m^2	IV
Mitoxantrone	10-15 mg/m^2	IV
Mitomycin C	10 mg/m^2	IV
Paclitaxel	175-200 mg/m^2	IV
	80 -100 mg/m^2	IV
Vinblastine	3-4 mg/m^2	IV
Vinorelbine	20-25 mg/m^2	IV

Among these agents, anthracycline, taxanes, vinorelbine, cisplatin, and 5-FU are more commonly used as combination regimens because of high activity and/or low toxicity. Four predictive markers selected in this study including Topo II, tau, ERCC1

and TS have been reported to predict the response of anthracycline, taxanes, cisplatin and 5-FU, respectively. These 4 markers are illustrated below.

1.5.1 Topo II

Anthracyclines are the most widely used chemotherapy for breast cancer. In the 1980s, it was shown that anthracyclines inhibit topoisomerase II α enzyme. Interaction with the topoisomerase II–DNA complex results in double-stranded DNA breaks and subsequently apoptosis. The gene that codes for topoisomerase II, which is the primary target for the anthracyclines, is *TOP2A.*   In a large adjuvant trial coordinated by the Danish Breast Cancer Cooperative Group (DBCG 89D), 980 pre and postmenopausal high-risk patients were randomized to receive either CEF (cyclophosphamide, epirubicin, and 5-fluorouracil) or CMF (cyclophosphamide, methotrexate, and 5-fluorouracil). A significant predictive value for anthracycline efficacy was found with respect to *TOP2A* amplification[8]. In addition, the predictive value of *TOP2A* gene aberrations has been investigated in a number of clinical studies, which, in total, include > 5,000 patients. All these studies seem to confirm the predictive value of *TOP2A* gene amplification[12-17].

Some studies using IHC to measure Topo II expression also showed that Topo II expression was associated with tumor response[12,13,16]. Although *TOPO2A* gene amplification by fluorescence in situ hybridization (FISH) is thought to be more specific than measuring expression by immunohistochemistry (IHC) for predicting anthracycline responsiveness, a previous study also showed that FISH may miss some tumors with biologically significant TOPO II over expression[12].

1.5.2 Tau

Tau is a microtubule associated protein, which promotes tubulin polymerization and stabilizes microtubule. Through a systemic pharmacogenomic approach, loss of Tau expression was found to be associated with taxane resistance[9]. In other two study, tau mRNA expression and Tau expression also showed to predict chemo-resistance in breast cancer and gastric cancer, respectively[18,19]. In-vitro study demonstrated that down-regulation of Tau increased sensitivity of breast cancer cells to paclitaxel by reducing paclitaxel induced microtubule polymerization[9].

1.5.3 ERCC1

The excision repair cross-complementation group 1 (ERCC1) enzyme plays a

rate-limiting role in the nucleotide excision repair pathway that recognizes and removes cisplatin-induced DNA adducts[20]. ERCC1 is also important in the repair of interstrand cross-links in DNA and in recombination processes. In vitro studies have linked platinum resistance to the expression of *ERCC1* messenger RNA (mRNA) in cell lines[21,22]. The relation between the expression of ERCC1 expression and resistance to platinum compounds has been corroborated by several clinical studies in patients with advanced gastric, ovarian, colorectal, esophageal, or non–small-cell lung cancer[23-27]. In addition, a large adjuvant study in non-small cell lung cancer showed that In the group of patients with ERCC1-negative tumors who received cisplatin based treatment, the risk of death was decreased by 35% (hazard ratio,0.65). By contrast, the risk of death was not decreased among patients with ERCC1-positive tumors who received cisplatin-based adjuvant chemotherapy (hazard ratio, 1.14)[11].

Recently, a prospective randomized phase III trial comparing standard treatment with docetaxel/ cisplatin versus individual treatment by *ERCC1* mRNA expression showed that individual arm achieved better response rate (50.7% vs. 39.3%, *P* =0.02). In this study, of 444 patients enrolled, 78 (17.6%) of 444 enrolled patients went off study before receiving one cycle of chemotherapy, mainly due to insufficient tumor tissue for ERCC1 mRNA assessment.[28].

1.5.4 TS (will be prospectively examined but not used for selection of chemotherapy)

5-FU is widely used in the treatment of a range of cancers, including colorectal and breast cancers, and cancers of the aerodigestive tract. The mechanism of cytotoxicity of 5-FU has been ascribed to the misincorporation of fluoronucleotides into RNA and DNA and to the inhibition of the nucleotide synthetic enzyme thymidylate synthase (TS). Preclinical studies have demonstrated that TS expression is a key determinant of 5-FU sensitivity. Gene amplification of TS with consequent increases in TS mRNA and protein has been observed in cell lines that are resistant to 5-FU and fluorodeoxyuridine (FUDR)[29,30].Multiple clinical investigations have measured TS expression by immunohistochemistry and reverse-transcription PCR (RT-PCR) and have shown an improved response to 5-FU-based therapy in patients with low tumoral TS expression[31-34]. In breast cancer, immunohistochemical analysis may be a better tool than analysis by RT-PCR in detection of TS, because of TS expression in cells besides carcinoma cells[35].

1.6 Other biomarkers

Other potential biomarkers that are not target of chemotherapy have been reported to predict chemotherapy response. These markers include P53, c-MYC and ATP-dependent transporter (ABCB1)[7,36,37]. In this study, we plan to validate the predictive power of these markers and their correlation with the 3 biomarkers mentioned above.

1.7 Trial rationale

By the evidence levels of 3 markers and efficacy and synergistic effects of 6 drugs, we develop a tailored therapy schedules (Summarized in Table 3).

Marker			Chemotherapy agent	
Topo II	Tau	ERCC1		
+	+	+	epirubicin	HDFL
+	+	-	epirubicin	cisplatin
+	-	+	docetaxel	epirubicin
+	-	-	docetaxel	epirubicin
-	+	+	vinorelbine	HDFL
-	+	-	vinorelbine	cisplatin
-	-	+	docetaxel	HDFL
-	-	-	docetaxel	cisplatin

Remarks: in case of undetermined result, Topo II undermined will be allocated as Topo II (+)；Tau undermined will be allocated as Tau (-)；ERCC1 undermined will be allocated as ERCC1 (-).

```
                                            ERCC1 +   ⟶   E-HDFL
                              Tau +
                                            ERCC1 -   ⟶   EP
               +
                              Tau -         ⟶             TE


      Topo II
                                            ERCC1 +   ⟶   N-HDFL
                              Tau +
                                            ERCC1 -   ⟶   NP
               -
                                            ERCC1 +   ⟶   T-HDFL
                              Tau -
                                            ERCC1 -   ⟶   TP
```
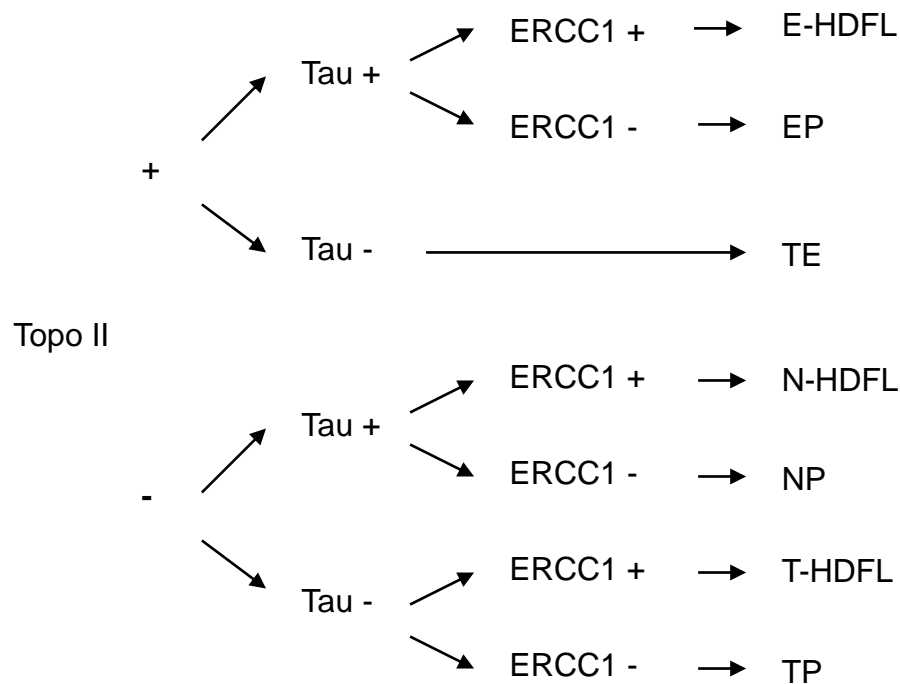
As mentioned earlier, pCR rate is now considered to be the most important study endpoint to assess the efficacy of neoadjuvant chemotherapy regimens. TE is considered as highly active regimen for breast cancer. Tailored therapy is potentially more active because selecting regimens by the biomarkers specific for chemotherapy drug may improve the efficacy. The randomized phase III study aims to compare the efficacy of TE regimen and 7 tailored regimens based on 3 predictive biomarkers and the primary endpoint is the pathological complete response rate.

We include vinorelbine and/or HDFL (weekly high dose 5FU/Leucovorin 24 hours infusion) to those tailored regimens which have only one or less than one of chemotherapy agent (i.e., docetaxel, epirubicin, cisplatin) could be selected according to biomarker marker study, to make each regimen as duplet chemotherapy regimen. The rationale of selecting vinorelbine and HDFL is as follows. Vinorelbine, a new semisynthetic vinca alkaloid, is well tolerated with significantly less neurotoxicity than other vinca alkaloids (vincristine and vinblastine) and a low incidence of subjective toxicities[38]. Vinorelbine, administered weekly as a single agent by an intravenous route, resulted in major objective response in about 45% of patients. Even in patients previously exposed to standard chemotherapy, 20–30% achieved a major objective response[4,39-41]. In one of our pilot studies, we used an HDFL regimen for the treatment of advanced breast cancer patients with heavily pretreated status or recurrence after high-dose chemotherapy with peripheral stem cells support. Even in this group of patients, an impressive response rate of 33% was noted. Further, an HDFL regimen

has repeatedly been demonstrated to cause minimal myelosuppression and therefore is an ideal component for combination chemotherapy with other cytotoxic agents against gastric cancer[2,42]. We also has reported a 70% response rate of vinorelbine plus HDFL regimen in advanced breast cancer.[43]

2. OBJECTIVES

2.1 Primary objective

- To evaluate and compare the pathological complete response (pCR) rates, mainly the primary tumor (T) pCR rate, after neoadjuvant chemotherapy with tailored chemotherapeutic regimens or TE for stage II/III breast cancer with tumor size more than 3 cm

2.2 Secondary objectives

- To evaluate the overall clinical response rate
- To evaluate the safety profiles

3. PATIENT SELECTION

3.1 Inclusion criteria

To be eligible for inclusion, each patient must fulfill all of the following criteria:

3.1.1   Patients must have a histologically confirmed invasive breast carcinoma.

3.1.2   The invasive breast cancer should be documented Her2/neu negative, including score 0, 1+, or 2+ by immunohistochemistry

3.1.3   Clinical stage of breast cancer should be T2-4, N0-3, M0, with tumor size more than 3 cm in greatest diameter measured by breast echo, CT scan or MRI.

3.1.4   No prior treatment (irradiation, chemotherapy, hormonal, immunotherapy or investigational, etc.) for invasive breast cancer excluding therapy for ductal carcinoma in situ (DCIS).

3.1.5   Subjects who received radiotherapy for DCIS may enroll.

3.1.6   ECOG performance status of 0-1

3.1.7   Laboratory parameter

    A.   Absolute neutrophil count (ANC) $\geqq$ 1500/mm^3

    B.   Total bilirubin $\leqq$ 2.0 times the upper limit of normal (ULM)

    C.   AST or ALT $\leqq$ 2.5 times the upper limit of normal (ULM)

    D.   Platelets $\geqq$ 100,000/mm^3

    E.   Serum creatinine $\leqq$ 1.5 x ULM

    F.   Fasting serum triglyceride $\geqq$ 70 mg/dL, which should be checked within

one week before entry into this study. The lower limit for fasting serum triglyceride (70 mg/dL) is set to avoid HDFL-related hyperammonemic encephalopathy, which occurs in around 5% of Taiwanese patients[43].

3.1.8　Patients must be women with age 20 years or older.

3.1.9　Disease free of prior malignancy for $\geqq$ 5 years with the exception of curatively treated non-melanoma skin cancer or cervical carcinoma in situ with a disease-free interval of at least 5 years.

3.1.10　Women of childbearing potential (WOCBP) must be using an adequate method of contraception to avoid pregnancy throughout the study and for up to 8 weeks after the last dose of chemotherapy. WOCBP must have a negative serum or urine pregnancy test within 7 days before the first dose of chemotherapy.

3.1.11　Patients must sign an informed consent form.

3.2 Exclusion criteria

3.2.1　Evidence of breast cancer metastatic other than axillary lymph nodes or inflammatory breast cancer

3.2.2　Bilateral breast cancer, metaplastic carcinoma, or mucinous carcinoma

3.2.3　Inadequate biopsy specimen for IHC study

3.2.4　Known allergy to any of the study drugs or to agents containing Cremophor

3.2.5　Serious intercurrent infections or medical illnesses that are uncontrolled or the control of which may be jeopardized by this therapy

3.2.6　Psychiatric disorders or other conditions regarding the subject incapable of complying with the requirements of the protocol

3.2.7　Evidence of baseline sensory or motor neuropathy

3.2.8　Other concurrent anti-tumor, chemotherapy, hormonal therapy, immunotherapy regimens or radiation therapy

3.2.9　WOCBP who are unwilling or unable to use an acceptable method to avoid pregnancy for the entire study period and for up to 8 weeks after the last dose or chemotherapy

3.2.10　Women who are currently pregnant or breast feeding

4. PLAN OF THE STUDY

4.1 Overall Study design

This is an open-label, multicenter randomized phase III clinical trial to compare the efficacy, mainly pathological complete response (pCR) rates, and safety after neoadjuvant chemotherapy with tailored chemotherapeutic regimens or TE for stage IIB/III breast cancer with tumor size more than 3 cm.

Patients will be randomized in a 1:1 ratio to receive either TE chemotherapy (control group) or tailored chemotherapy group. If patient is randomized to the tailored chemotherapy arm, the actual treatment given to individual patients will be determined by the result of immunohistochemical staining result of patient's tumor biopsy sample. The treatment are summary as followed, and regimens details are described on section 7 :

Groups	IHC results	Regimens
Control chemotherapy	**Any**	**TE**
Tailored chemotherapy	Tau + topo II + ERCC1 +	**E-HDFL**
	Tau + topo II + ERCC1 –	**EP**
	Tau + topo II – ERCC1 +	**N-HDFL**
	Tau + topo II – ERCC1 –	**NP**
	Tau – topo II + ERCC1 + or –	**TE**
	Tau – topo II – ERCC1 +	**T-HDFL**
	Tau – topo II – ERCC1 –	**TP**

4.2 Number of patients

Approximately 316 patients will be enrolled in order to obtain a total of 272 evaluable patients, with 136 evaluable patients in each arm.

4.3 Study schedule

Expected recruitment rate: 14 patients/month
Duration of the recruitment period: 24 months
Duration of the study: 28 months

4.4 Method of assigning patients to treatment groups

Patient eligibility will be established before treatment randomization.

All enrolled subjects will be allocated an I-code irrespective of whether they are subsequently randomized to receive study regimens. The I-code is a 5 digit number made of the center number (first 2 digits) and the subject number (last 3 digits) within that particular center e.g. the first patient enrolled at center number 03 would be allocated the I-code I03001, the second patient enrolled at that center would be allocated the I-Code I03002 and so on. This number is the subject's unique identifier and is used to identify the subject on the CRF.

Subject will be randomized strictly sequentially, as patients are considered eligible.

Clinical trial center of National Taiwan University Hospital will produce a center-specific labeled randomization. Patients will be randomized in a 1:1 ratio. The randomization scheme will be generated by biostatistics and produced by a computer software program that incorporates a standard procedure for generating random numbers. The randomization number will be stratified by ER status (ER+ vs ER-), and clinical primary tumor stage (T2 vs T3/T4). If a subject discontinues from the study, the I-code number and subject number will not be reused, and the patient will not be allowed to re-enter the study.

When the subjects are randomized to the Tailored Chemotherapy group, the specific treatment regimens will be selected according to the result of IHC study of biopsy tumor tissues.

4.5 Treatment Duration

Each patient will receive four cycles of neoadjuvant chemotherapy before surgery unless disease progression is documented or unacceptable toxicity occurs.

4.6 Concomitant Treatment

Concomitant medication allowed and not permitted is described below:

Allowed:
- Ancillary treatments for medical indication are permitted.
- Antiemetics, antiallergic measures are permitted.
- G-CSF is permitted, either for prophylactic use or febrile neutropenia.
- Preventive oral or i.v. antibiotics when neutrophils $< 0.5 \times 10^9$/l without fever are recommended but the decision to use antibiotics in this case will be left to the current policy within the different hospitals.

Not permitted:
The patients should not receive other investigational drugs or anticancer treatment while on study.

4.7 Discontinuation of protocol treatment

A patient will be discontinued protocol treatment from the study under the following circumstances

- Having completed the assigned treatment and observations
- Objective disease progression
- Chemotherapy delay longer than 3 weeks

- Patient wishes to withdraw from this study at her own request.
- Patients develop any condition of the exclusion criteria
- Patients with poor compliance
- Investigator feels that patient withdrawal is better choice for patients
- Losing the patient to follow-up (i.e., dropping out)
- Administration of radiotherapy, non-protocol chemotherapy, or an experimental drug during protocol treatment

5. TREATMENT

5.1 Materials and Supplies

Commercially available docetaxel (Taxotere , Sanofi-Aventis), epirubicin, cisplatin, vinorelbine (Navelbine, 台灣友華) and 5-FU will be used for this study.

5.2 Dose Calculation and Administration Routes

Body surface area calculations are based on the patient's actual body weight. Calculate the body surface area of the patient according to actual height and weight at the beginning of each cycle. For safety and convenience of drug administration, a Port-A catheter implantation or PICC is needed before the start of the first chemotherapy course if patient is to be treated as out-patient basis

5.3 Concomitant Treatment

No other chemotherapy, immunotherapy, hormonal therapy, radiation therapy, or experimental medications will be permitted while the patients are on the study treatment. Any disease progression requiring other forms of specific anti-tumor therapy will be cause for early discontinuation in this study. Antiemetics including 5-HT3 antagonists, glucocorticoids, metoclopramide and aprepitant are permitted before and after chemotherapy. The use of two doses of prophylaxis G-CSF (2 mcg/kg s.c. or 5 mcg/kg i.v. per day x 2 days) in each cycle of chemotherapy is sponsored by this trial. However, the usage and doses of prophylaxis G-CSF is at investigator's discretion.

5.4 TE regimen

Treatment schedule will be as follows:

- Epirubicin 45 mg/m^2 on day 1 and day 8
- Docetaxel 35 mg/m^2 on day 1 and day 8
- Lenograstim (G-CSF) 2 mcg/kg s.c. or 5 mcg/kg i.v. per day on day 10 and day 11 (i.e., started 2 days after day 8 chemotherapy)

Cycled every 21 days

5.4.1 **Administration:**

Epirubicin should be infused over 5 to 30 minutes. Longer duration of infusion is allowed if using central venous line (port A, CVP, or PICC). The assessing for patency frequently during the infusion and maintaining extravasation precautions throughout the infusion is warranted. For prevention of epirubicin related hyper-emesis pretreatment with granisetron (Kytril) (3 mg/vial) IV or other 5-HT$_3$ antagonist will be given.

Docetaxel should be administered by 1-hour intravenous infusion in this study. To avoid docetaxel-related hypersensitivity reactions and edema, all patients should receive prophylactic pre- and post-medication of oral dexamethasone 8 mg bid (or i.v. equivalent potent steroid with same duration) for 3 days. Close monitoring of the patient's vital signs is indicated.

Each center can use their own anti-emetics and hydration regimen as per their center's own guideline.

Prophylaxis G-CSF (lenograstim 2 mcg/kg s.c. or 5 mcg/kg s.c. per day)   could be given on day 10, and day 11. If the day 8 chemotherapy is delayed, the G-CSF could be started on the 3rd day of day 8 chemotherapy. However, the use of prophylaxis G-CSF is at investigator's discretion.

This regimen will be repeated every 21 days as a treatment course. If the day 8 chemotherapy of previous cycle is delayed, next cycle of chemotherapy should be started at lease 12 days after day 8 dose.

5.5 E-HDFL regimen

Treatment schedule will be as follows:

> - Epirubicin 45 mg/m^2 on day 1 and day 8
> - 5-FU 2000 mg/m^2 and leucovorin 300 mg/m^2 , **24-hour infusion** on day 1 and day 8
> - Lenograstim (G-CSF) 2 mcg/kg s.c. or 5 mcg/kg i.v. per day on day 10 and day 11 (i.e., started 2 days after day 8 chemotherapy)
> Cycled every 21 days

5.5.1 **Administration:**

Epirubicin should be infused over 5 to 30 minutes. Longer duration of infusion is allowed if using central venous line (port A, CVP, or PICC).The assessing for patency frequently during the infusion and maintaining extravasation precautions throughout the infusion is warranted. For prevention of epirubicin related hyper-emesis pretreatment with granisetron (Kytril) (3 mg/vial) IV or other 5-HT antagonist will be given.

5-FU in combination with leucovorin should be administered in 500 mL of normal saline, **24-hrs continuous infusion** by infusion pump. The assessing for **ensuring the speed of the infusion is warranted.**

Each center can use their own anti-emetics and hydration regimen as per their center's own guideline.

Prophylaxis G-CSF (lenograstim 100 mcg/vial or 250 mcg/vial) 2 mcg/kg s.c or 5 mcg/kg s.c. could be given on day 10, and day 11. If the second dose (day 8) chemotherapy is delayed, the G-CSF could be started 2 days after second dose chemotherapy. However, the use of prophylaxis G-CSF is at investigator's discretion.

This regimen will be repeated every 21 days as a treatment course. If the day 8 chemotherapy of previous cycle is delayed, next cycle of chemotherapy should be started at lease 12 days after day 8 dose.

5.6 EP regimen

Treatment schedule will be as follows:

> - Epirubicin 45 mg/m^2 on day 1 and day 8
> - Cisplatin 35 mg/m^2 on day 1 and day 8
> - Lenograstim (G-CSF) 2 mcg/kg s.c. or 5 mcg/kg s.c. per day on day 10 and day 11 (i.e., started 2 days after day 8 chemotherapy)
> Cycled every 21 days

5.6.1 **Administration:**

Epirubicin should be infused over 5 to 30 minutes. Longer duration of infusion is allowed if using central venous line (port A, CVP, or PICC). The assessing for patency frequently during the infusion and maintaining extravasation precautions throughout the infusion is warranted. For prevention of epirubicin related hyper-emesis pretreatment with granisetron (Kytril) (3 mg/vial) IV or other 5-HT antagonist will be given.

Cisplatin should be administered in 500 mL of normal saline. 24-hrs continuous infusion is recommended in this study. However, infusion for more than 3 hours is also acceptable. For prevention of cisplatin related hyper-emesis pretreatment with granisetron (Kytril) (3 mg/vial)or other 5-HT3 antagonist IV will be given. For prevention delay type emesis, oral granisetron 1mg/tab 1 tab bid or other 5-HT3 antagonist on day 2-5 is optional at the investigators discretion.

Each center can use their own anti-emetics and hydration regimen as per their center's own guideline. It is not mandatory to give extra hydration for patients receiving cisplatin 24 hours infusion. However, in case of shorter infusion duration (3-6 hours), aggressive hydration with more than 3000 cc with or without lasix injection is warranted.

Prophylaxis G-CSF (lenograstim 100 mcg/vial or 250 mcg/vial) 2 mcg/kg s.c., or 5 mcg/kg s.c. could be given on day 10, and day 11. If the second dose (day 8) chemotherapy is delayed, the G-CSF could be started 2 days after second dose chemotherapy. However, the use of prophylaxis G-CSF is at investigator's discretion.

This regimen will be repeated every 21 days as a treatment course. If the day 8 chemotherapy of previous cycle is delayed, next cycle of chemotherapy should be started at lease 12 days after day 8 dose.

5.7 N-HDFL regimen

Treatment schedule will be as follows:

- Navelbine 25 mg/m^2 on day 1 and day 8
- 5-FU 2000 mg/m^2 and leucovorin 300 mg/m^2 , **24-hour infusion** on day 1 and day 8
- Lenograstim (G-CSF) 2 mcg/kg sc or 5 mcg/kg s.c. per day on day 10 and day 11 (i.e., started 2 days after day 8 chemotherapy)
Cycled every 21 days

5.7.1 **Administration:**

The use of a central venous line (port A, PICC, or CVP) is recommended for safe administration due to the local toxicity of Navelbine. Navelbine should be infused over 5 to 30 minutes. The assessing for patency frequently during the infusion and maintaining extravasation precautions throughout the infusion is warranted.

5-FU in combination with leucovorin should be administered in 500 mL of normal saline, **24-hrs continuous infusion** by infusion pump. The assessing for **ensuring the speed of the infusion is warranted**.

Each center can use their own anti-emetics and hydration regimen as per their center's own guideline. However, it is not mandatory to give extra hydration for patients receiving this protocol.

Prophylaxis G-CSF (lenograstim 100 mcg/vial or 250 mcg/vial) 2 mcg/kg s.c. or 5 mcg/kg s.c. per day x 2 days, could be given on day 10, and day 11. If the second dose (day 8) chemotherapy is delayed, the G-CSF could be started 2 days after second dose chemotherapy. However, the use of prophylaxis G-CSF is at investigator's discretion.

This regimen will be repeated every 21 days as a treatment course. If the day 8 chemotherapy of previous cycle is delayed, next cycle of chemotherapy should be started at lease 12 days after day 8 dose.

5.8 NP regimen

Treatment schedule will be as follows:

- Navelbine 25 mg/m^2 on day 1 and day 8 - Cisplatin 35 mg/m^2 on day 1 and day 8 - Lenograstim (G-CSF) 2 mcg/kg sc or 5 mcg/kg sc per day on day 10 and day 11 (i.e., started 2 days after day 8 chemotherapy) Cycled every 21 days

5.8.1 **Administration:**

The use of a central venous line is recommended for safe administration due to the local toxicity of Navelbine. Navelbine should be infused over 5 to 30 minutes. The assessing for patency frequently during the infusion and maintaining extravasation precautions throughout the infusion is warranted.

Cisplatin should be administered in 500 mL of normal saline. 24-hrs continuous infusion is recommended in this study. However, infusion for more than 3 hours is also acceptable. For prevention of cisplatin related hyper-emesis pretreatment with granisetron (Kytril) (3 mg/vial) or other 5-HT3 antagonist IV will be given. For prevention delay type emesis, oral granisetron 1mg/tab 1 tab bid or other 5-HT3 antagonist on day 2-5 is optional at the investigators discretion.

Each center can use their own anti-emetics and hydration regimen as per their center's own guideline. It is not mandatory to give extra hydration for patients receiving cisplatin 24 hours infusion. However, in case of shorter infusion duration (3-6 hours), aggressive hydration with more than 3000 cc with or without lasix injection is warranted.

Prophylaxis G-CSF (lenograstim 100 mcg/vial or 250 mcg/vial), 2 mcg/kg sc or 5 mcg/kg sc per day x 2 days, could be given on day 10, and day 11. If the second dose (day 8) chemotherapy is delayed, the G-CSF could be started 2 days after second dose chemotherapy. However, the use of prophylaxis G-CSF is at investigator's discretion.

This regimen will be repeated every 21 days as a treatment course. If the day 8 chemotherapy of previous cycle is delayed, next cycle of chemotherapy should be started at lease 12 days after day 8 dose.

5.9 T-HDFL regimen

Treatment schedule will be as follows:

- Docetaxel   35 mg/m^2 on day 1 and day 8 - 5 Fluorouracil 2000 mg/m^2 and leucovorin 300 mg/m^2 , **24-hour infusion** on day 1 and day 8 - Lenograstim (G-CSF) 2 mcg/kg sc or 5 mcg/kg sc per day on day 10 and day 11 (i.e., started 2 days after day 8 chemotherapy) Cycled every 21 days

Docetaxel should be administered by 1-hour intravenous infusion in this study. To avoid docetaxel-related hypersensitivity reactions and edema, all patients should receive prophylactic pre- and post-medication of oral dexamethasone 8 mg bid (or i.v. equivalent potent steroid with same duration) for 3 days. Close monitoring of the patient's vital signs is indicated.

5-FU in combination with leucovorin should be administered in 500 mL of normal saline, **24-hrs continuous infusion** by infusion pump. The assessing for **ensuring the speed of the infusion is warranted**.

Each center can use their own anti-emetics and hydration regimen as per their center's own guideline. However, it is not mandatory to give extra hydration for patients receiving this protocol.

Prophylaxis G-CSF (lenograstim 100 mcg/vial or 250 mcg/vial), 2 mcg/kg sc or 5 mcg/kg sc per day, could be given on day 10, and day 11. If the second dose (day 8) chemotherapy is delayed, the G-CSF could be started 2 days after second dose chemotherapy. However, the use of prophylaxis G-CSF is at investigator's discretion.

This regimen will be repeated every 21 days as a treatment course. If the day 8 chemotherapy of previous cycle is delayed, next cycle of chemotherapy should be started at lease 12 days after day 8 dose.

5.10 TP regimen

Treatment schedule will be as follows:

- Docetaxel   35 mg/m^2 on day 1 and day 8 - Cisplatin 35 mg/m^2 on day 1 and day 8 - Lenograstim (G-CSF) 2 mcg/kg sc or 5 mcg/kg sc per day on day 10 and day 11 (i.e., started 2 days after day 8 chemotherapy) Cycled every 21 days

Docetaxel should be administered by 1-hour intravenous infusion in this study. To avoid docetaxel-related hypersensitivity reactions and edema, all patients receive prophylactic pre-medication of oral dexamethsone 8 mg bid (or i.v. equivalent potent steroid with same duration) for 3 days. Close monitoring of the patient's vital signs is indicated.

Cisplatin should be administered in 500 mL of normal saline. 24-hrs continuous infusion is recommended in this study. However, infusion for more than 3 hours is also acceptable. For prevention of cisplatin related hyper-emesis pretreatment with granisetron (Kytril) (3 mg/vial) or other 5-HT3 antagonist IV will be given. For prevention delay type emesis, oral granisetron 1mg/tab 1 tab bid or other 5-HT3 antagonist on day 2-5 is optional at the investigators discretion.

Each center can use their own anti-emetics and hydration regimen as per their center's own guideline. It is not mandatory to give extra hydration for patients receiving cisplatin 24 hours infusion. However, in case of shorter infusion duration (3-6 hours), aggressive hydration with more than 3000 cc with or without lasix injection is warranted.

Prophylaxis G-CSF (lenograstim 100 mcg/vial or 250 mcg/vial), 2 mcg/kg sc or 5 mcg/kg sc per day could be given on day 10, and day 11. If the second dose (day 8) chemotherapy is delayed, the G-CSF could be started 2 days after second dose chemotherapy. However, the use of prophylaxis G-CSF is at investigator's discretion.

This regimen will be repeated every 21 days as a treatment course. If the day 8 chemotherapy of previous cycle is delayed, next cycle of chemotherapy should be started at lease 12 days after day 8 dose.

6.   CHEMOTHERAPY DELAY AND DOSE MODIFICATION

Chemotherapy delay or dose modification is made according to the greatest degree of toxicity that is graded base on Common Terminology Criteria for Adverse Events v3.0 (CTCAE).

Day 1 treatment of each cycle may be delayed no more than 3 weeks to allow recovery from acute toxicity, or patient should be failure from regimen. Day 8 treatment may be delayed no more than 1 week to allow recovery from acute toxicity, or will be omitted for that cycle.

6.1 Hematologic toxicity

The following dose adjustments are based on the hematologic counts on the day of or one day before treatment.

6.1.1   Neutropenia and/or its complications

	WBC or ANC (x 10^9/L)	Action to be taken
for day 8 chemotherapy	WBC $\geqq$ 2.5 and ANC $\geqq$ 1.0	Treat on time
	WBC < 2.5 or ANC < 1.0	Delay and check WBC at least twice per week. If WBC $\geqq$ 2.5 and ANC $\geqq$ 1.0, then re-start treatment without modification.

for day 1 chemotherapy	WBC ≧ 3.0 and ANC ≧1.5	Treat on time
	WBC < 3.0 or ANC < 1.5	Delay and check WBC at least twice per week. If WBC ≧ 3.0 and ANC ≧ 1.5, then re-start treatment without modification. May consider increase doses and days of G-CSF treatment after chemotherapy thereafter.

6.1.2 **Thrombocytopenia**

	Platelet Count (/μL)	Action to be taken
for each dose of chemotherapy	≧75,000	Treat on time
	< 75,000	Delay and check platelet twice per week

6.2 Non-Hematologic toxicity

6.2.1 **Peripheral Neuropathy**

	Grade of neurological toxicity at the time of planned treatment	Action to be taken
Docetaxel , cisplatin, and navelbine containing regimen i.e. TE, N-HDFL, NP, TP, T-HDFL,	Grade 0 or 1	Treat on time
	Grade 2 or 3	Delay chemotherapy doses by one week. If patient recovers to grade 0/1 toxicity, then start treatment with 25% reduction for docetaxel , cisplatin, and navelbine. If not recovered to grade 0/1 in three weeks, patients is failure from regimen for excessive toxicity. Salvage chemotherapy or direct operation could be given at investigators discretion.
	Grade 4	Patient is discontinued from study.

6.2.2 **Gastrointestinal Toxicity**

If ≧grade 3 mucositis occurs, chemotherapy should be withheld until resolution to ≦ grade 1.

In case of diarrhea ≧grade 3, chemotherapy should be withheld until resolution to ≦ grade 1.

Nausea and/or vomiting should be controlled with adequate Antiemetics.

6.2.3 **Elevation of Liver Function Tests**

The following dose adjustments are based on results derived within 3 days of each treatment.

If SGOT/SGPT $\leqq$ 5 x UNL (upper normal limit) and bilirubin $\leqq$ 3 x UNL, chemotherapy will be given without dose modification.

If SGOT/SGPT > 5 x UNL or bilirubin > 3 x UNL occurs, dose delay by a maximum of 3 weeks. If liver toxicity persists for more than 3 weeks, patient should be failure from regimen.

6.2.4 **Renal Toxicity**

Dose modifications are based on results derived within 3 days of each treatment.

Calculated creatinine clearance mL/min	Cisplatin dose to be administered	5-FU dose to be administered	Docetaxel , navelbine, or epirubicin to be administered
>50	No change	No change	No change
50-30	50% reduction	25% reduction	No change
<30	Discontinuation	Discontinuation	Discontinuation

CrCl = (140 – age in years) x weight in kg x 0.85(female)
          72 x serum creatinine in mg/dl

6.2.5 **Other Non-hematological Toxicity**

If toxicities are $\leqq$ grade 2, manage symptomatically if possible, and treat without dose change.

If toxicity are $\geqq$ grade 3, treatment should be withheld (except for alopecia) until resolution to grade 1 or baseline if baseline was greater than grade 1.

7. RE-EVALUATION AND MANAGEMENT AFTER NEOAJUVANT CHEMOTHERAPY

7.1 After each cycle of first 3 cycles

Patients should receive objective evaluation of tumor response by physical examination after every course of treatment or as clinical indicated. Patients with objective response to chemotherapy by physical examination will continue receive original assigned chemotherapeutic regimens. In patients with suspicious PD (progression disease) by physical examination after any cycle of treatment,

objective evaluation of tumor response by image study should be arranged. In patients with tumor size dose not decrease in size by physical examination after 2 cycle of chemotherapy, objective evaluation of tumor response by image study should also be arranged. In patient with tumor size less than 4 cm, tumor size could be measured by breast echo or CT scan, breast MRI. In patient with tumor size larger than 4 cm, tumor size should be measured by CT scan or breast MRI. It is recommended to apply same modality of image system to evaluate the tumor size before and after treatment.

7.1.1 **Patient with tumor progression**

Patients with PD or SD (stable disease) with tumor size increase 1 to 19 % by RECIST (Response evaluation criteria in solid tumor) criteria after any cycles of chemotherapy treatment will receive salvage chemotherapy or operation at the investigator's discretion. If patient is allocated in control chemotherapy group, the result of tumor tissue IHC study will be released to the investigator for his information.

7.1.2 **Patient without tumor progression**

Patients with clinical CR (complete response), PR (partial response) or SD with tumor size decrease 0 to 29% by RECIST criteria after image study will receive continue courses of original assigned chemotherapy regimen until at least a total of four cycles of chemotherapy are given.

7.2 After four cycles

After a total of four cycles of chemotherapy, patients should receive objective evaluation of tumor response by image study. In patient with tumor size less than 4 cm, tumor size could be measured by breast echo or CT scan, breast MRI. In patient with tumor size larger than 4 cm, tumor size should be measured by CT scan or breast MRI. It is recommended to apply same modality of image system to evaluate the tumor size before and after treatment.

Then, patient will receive operation for tumor resection, or continued neoadjuvant chemotherapy with same or different regimens at the investigator's discretion.

7.2.1 **In patients who received tumor resection after 4 cycles of neoadjuvant chemotherapy**

For patient with clinical stage II breast cancer, whether to give additional adjuvant chemotherapy is at the investigator's discretion. For patient with clinical stage III, additional 2 to 4 cycles of adjuvant chemotherapy is recommended. However, the choice of regimens and number of cycles of adjuvant chemotherapy will be

decided at the investigator's discretion. In patients who are allocated in control chemotherapy group, the result of tumor tissue IHC study will be released to the investigator for his information. Suggested adjuvant chemotherapy protocols for patients received different neoadjuvant chemotherapy are listed in section 7.3.

7.2.2   **In patient who will receive continued neoadjuvant chemotherapy**
In patient who will receive continued neoadjuvant chemotherapy: **tumor biopsy is required** in patients with residual tumor smaller than 3 cm, in order to prove that residual invasive tumor still exists. There is no need to continue neoadjuvant chemotherapy if no evidence of residual invasive tumor exists after 4 cycles neoadjuvant chemotherapy. After operation, patient may receive additional adjuvant chemotherapy. The decision of whether to give additional adjuvant chemotherapy, the choice of regimens and number of cycles of adjuvant chemotherapy is at the investigator's discretion. In patients who are allocated in control chemotherapy group, the result of tumor tissue IHC study will be released to the investigator for his information. Suggested chemotherapy protocols as additional neoadjuvant chemotherapy are listed in section 7.3.

7.3 Additional chemotherapy protocols

After 4 cycles of protocol neoadjuvant treatment, or after progression at any cycles of protocol neoadjuvant chemotherapy, patient may receive additional chemotherapy. Suggested chemotherapy regimens are listed in 7.3.1 and 7.3.2. If patient's tumor is Her2 IHC 2+, FISH study for Her2 gene amplification is recommended. In patient with Her2 FISH positive, treatment with trastuzumab alone or incorporate to chemotherapy regimen may be considered. However, the choice of regimens and number of cycles of adjuvant chemotherapy will be decided at the investigator's discretion.

Response	Suggestion of chemotherapy
pCR	No more chemotherapy, or additional 2 cycles of original regimen
PR	In patient with TE (taxotere plus epirubicin) treatment: No more chemotherapy, or additional 2 cycles of original regimen, or change to other regimen for 2-4 cycles In patient with other regimen treatment:

	Addition of 2-4 cycles of adjuvant chemotherapy containing epirubicin or taxotere, or classical CMF
SD	In patient with TE (taxotere plus epirubicin) treatment: No more chemotherapy, or additional 2 cycles of original regimen, or change to other regimen for 2-6 cycles In patient with treatment other than TE: Addition of adjuvant chemotherapy containing epirubicin or taxotere, or classical CMF for 2-6 cycles.
PD	In patient with TE (taxotere plus epirubicin) treatment: change to other regimen for 2-6 cycles In patient with treatment other than TE: Adjuvant chemotherapy should include epirubicin or taxotere, or classical CMF.

7.3.1 Post TE regimen (either in control arm or in tailored arm)

IHC marker results			Suggested other protocols
TopoII	**Tau**	**ERCC1**	
+	+	+	**CMF, N-HDFL, NG**
+	+	-	**CMF, NP, N-HDFL**
+	-	+	**CMF, N-HDFL, NG**
+	-	-	**CMF, NP, N-HDFL**
-	+	+	**CMF, N-HDFL, NG**
-	+	-	**CMF, NP, N-HDFL**
-	-	+	**CMF, N-HDFL, NG**
-	-	-	**CMF, NP, N-HDFL**

7.3.2 In tailored regimen arm (other than TE regimen)

Original	IHC marker result			Suggested other protocols
regimen	TopoII	Tau	ERCC1	
E-HDFL	+	+	+	TC, TP, CMF
EP	+	+	-	TC, CMF
N-HDFL	-	+	+	EC, CEF, TE, TC, TP, CMF
NP	-	+	-	CEF, TE, TC, T-HDFL, CMF
T-HDFL	-	-	+	EC, CEF, CMF
TP	-	-	-	CEF, EC, CMF

7.3.3 Representative regimens

CEF (or FEC)	Cyclophosphamide 500 mg/m^2 IV day1
	Epirubicin 90 or 100 mg/m^2 IV day1
	5-Fluorouracil 500 mg/m^2 IV day1
	Cycled every 21 days
	or
	Cyclophosphamide 600 mg/m^2 IV day1
	Epirubicin 75 mg/m^2 IV day1
	5-Fluorouracil 600 mg/m^2 IV day1
	Cycled every 21 days
	or
	Cyclophosphamide 75 mg/m^2 PO days 1-14
	Epirubicin 60 mg/m^2 IV day1 & 8
	5-Fluorouracil 500 mg/m^2 IV day1 & 8
	With prophylaxis oral antibiotics
	Cycled every 28 days
CMF	Cyclophosphamide 100 mg/m^2 PO days 1-14
	Methotrexate 40 mg/m^2 IV day1 & 8
	5-Fluorouracil 500 mg/m^2 IV day1 & 8
	Cycled every 28 days
EC	Cyclophosphamide 600 mg/m^2 IV day1
	Epirubicin 75 - 100 mg/m^2 IV day1

	Cycled every 21 days
NC	Navelbine 25 mg/m^2 IV day 1 & 8
	Cyclophosphamide 600 mg/m^2 IV day1
	Cycled every 21 days
NG	Navelbine 25 mg/m^2 IV day 1 & 8
	Gemcitabine 1000 mg/m^2 IV infusion (30mins) day 1 & 8
	Cycled every 21 days
N-HDFL	Navelbine 25 mg/m^2 IV day 1 & 8
	5-Fluorouracil 2600 mg/m^2 and leucovorin 300 mg/m2, **24-hour infusion** day 1 & day 8
	Cycled every 21 days
NP	Navelbine 25 mg/m^2 IV day 1 & 8
	Cisplatin 35 mg/m^2 IV infusion (4~24 hrs) day 1 & 8
	Cycled every 21 days
TE	Epirubicin 45 mg/m^2 IV day 1 & 8
	Docetaxel 35 mg/m^2 IV day 1 & 8
	Cycled every 21 days
TC	Docetaxel 60 - 70 mg/m^2 IV day 1
	Cyclophosphamide 600 mg/m^2 IV day 1
	Cycled every 21 days
T	Docetaxel 100 mg/m^2 IV day 1
	With or without prophylaxis GCSF
	Cycled every 21 days
T-HDFL	Docetaxel 35 mg/m^2 IV day 1 & 8
	5-Fluorouracil 2000 mg/m^2 and leucovorin 300 mg/m2, **24-hour infusion** day 1 & day 8
	Cycled every 21 days
TP	Docetaxel 35 mg/m^2 IV day 1 & 8
	Cisplatin 35 mg/m^2 IV infusion (4~24 hrs) day 1 & 8
	Cycled every 21 days

7.4 After complete chemotherapy

After complete chemotherapy (neoadjuvant plus adjuvant) and surgery, adjuvant

radiotherapy should be given in patients with originally clinical tumor size more than 5 cm (by CT scan or MRI), or in patients who receive breast conserving surgery. In patients with originally clinical tumor size less than 5 cm, adjuvant radiotherapy is suggested if metastatic axillary's lymph node is suspected according to image study (breast echo, CT scan, or MRI), unless the tissue diagnosis (lymph node aspiration cytology or biopsy) show negative result before chemotherapy was given. However, in patients with originally clinical tumor size less than 5 cm, the decision of whether to give adjuvant radiotherapy or not is at the investigator's discretion.

Adjuvant hormonal therapy will be given in patients with positive hormonal receptor finding.

8. PREPARATION OF TUMOR SAMPLES AND DETERMINATION OF THE IHC

8.1 Preparation

Part of paraffin embedded tumor blocks of primary tumor, preferentially with low amount of necrosis will be collected from every enrolled patients. The tumor blocks will be used to create tissue sections for immunohistochemical staining study.

8.2 Method of immunohistochemistry

Immunohistochemical studies will be performed on paraffin sections using an indirect biotin-avidin method. Sections cut at 4 µm thickness will be deparaffinized and rehydrated. Endogenous peroxidase activity will be blocked with hydrogen peroxide/methanol, and antigen retrieval will be performed in commercial buffer (Trilogy, Cell Marque, Hot Springs, AR, USA) by autoclave for 10 minutes. All primary antibodies will be incubated at a temperature of 4 ℃ overnight. Antibody reactivity was detected using the Ventana iView DAB Detection System (Nexus IHC, Ventana Medical Systems, Tucson, AZ, USA). Tissues known to express the determinants of interest were used as positive controls. The following mouse monoclonal antibodies were used:

Anti-ERCC1: mouse, clone 8F1, Neomarkers; 1: 50 dilution

Anti-tau: clone T1029, United States Biological, Swampscott, MA; 1: 20 dilution

Anti-topo II: Clone JH2.7; Neomarkers, Union City, CA; 1:20

8.3 Positivity definition

8.3.1 ERCC1 score: IHC score 0, no staining; 1, less staining than reference; 2,

similar to reference; 3, more intense than reference (reference: epithelial cells in tonsil control tissue and assigned an intensity of 2).

Positivity of ERCC1: tumors with a staining intensity score of 2 and with 50% or more positive nuclei or with a staining intensity score of 3 and 10% or more positive nuclei

8.3.2   Tau score : IHC score 0, no staining; 1, less staining than normal epithelium; 2, similar to normal epithelium; 3, uniform staining more   intense than normal cells.

Positivity of tau : IHC score 2 or 3 staining were considered tau positive.

8.3.3   Topo-II: Staining will be considered positive if at least 10% of the cells showed positive nuclear staining.

9. ASSESSMENT SCHEDULE

Patients will be screened before entry to establish eligibility.

Flow sheet of assessments

Tests and procedures	Screening		D-1 to D1 and D7 to D8 while on Rx	Every cycle while on Rx	After 4th cycles	At time of progression
	D-28 to 0	D-7 to 0				
Informed consent	X					
History and exam, wt, PS (ECOG)[1]		X		X		X
Serum pregnancy test[2]		X				
Hematology (CBC/DC)		X	X			
Biochemistry[3]		X		X		X
Triglyceride		X				
Chest x-ray		X			X	X
Tumor assessment by physical exam		X		X		X
Tumor assessment by image study[4]	X				X	X
Systemic staging work-up[5]	X					X
Tumor biopsy for IHC studies	X					

1. ECOG performance status scale.
2. For women of childbearing potential only.
3. Biochemistry includes Alb, Globulin, BUN, creatinine, bilirubin, AST, ALT, LDH, ALP, UA, Na, K, Ca and fasting sugar and triglyceride.
4. Tumor assessment: see section 7. In patient with tumor size less than 4 cm, tumor size could be measured by breast echo or CT scan, breast MRI. In patient with tumor size larger than 4 cm, tumor size should be measured by CT scan or breast MRI.
5. In patient with clinical stage III disease, bone scan and chest/abdominal CT scan or PET scan are needed. In patient with clinical stage II disease, the systemic image work up is optional.

10. MEASUREMENT OF THERAPEUTIC EFFECTS

The tumor response will be determined by applying RECIST criteria.

10.1 Measurability of tumor lesions at the baseline

10.1.1 Definition:

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter $\geqq$ 20 mm using conventional techniques or $\geqq$ 10 mm with spiral CT scan.

Non-measurable lesions - all other lesions, including small lesions (longest diameter < 20 mm with conventional techniques or < 10 mm with spiral CT scan) and inflammatory breast disease.

All measurements should be taken and recorded in metric notation, using a ruler or callipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Clinical lesions including skin nodules and palpable lymph nodes will be considered measurable when they are superficial. For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

10.1.2 Methods of Measurement

CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm.

Ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes and subcutaneous lesions. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination. In this study, tumor with less than 4 cm is allowed to be measured by breast echo.

10.1.3 Baseline documentation of "Target" and "Non-Target" lesions

All measurable lesions up to a maximum of five lesions in total should be identified

as target lesions and recorded and measured at baseline.

Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).

A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.

All other lesions (or sites of disease) should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

10.2 Response Evaluation

10.2.1 Response Criteria

Evaluation of target lesions

* Complete Response (CR): Disappearance of all target lesions
* Partial Response (PR):   At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
* Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started
* Stable Disease (SD):   Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started

Evaluation of non-target lesions

* Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level
* Stable Disease (SD):   Response/ Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits
* Progressive Disease (PD): Unequivocal progression of existing non-target lesions [1]

(1) Although a clear progression of "non- target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

10.2.2 **Evaluation of best overall response**

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target lesions	Non-target lesion	New lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.

10.2.3 **Confirmation**

According to RECIST criteria, to be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. In this study, extra early image study (other than protocol design) is not mandatory if there is no clinical disease progression. In patients who receive surgical resection within 4 weeks after the criteria for response are first met, the status of response will be assigned as the response already been confirmed.

10.3 Definition of pathological response

Pathological evaluation of tumor will be done by a pathologist who was blinded to the treatment received by the patient. Complete tissue samples (including primary breast tissue and axillary's lymph nodes) should be included for analysis and the

response was registered as follows: Patients with no residual viable invasive tumor cells in the surgical specimen (primary tumor and lymph nodes) will be classified as having a pathological *complete response* (pCR). Patients with no residual viable invasive tumor cells in the primary tumor will be classified as having a T-pCR. Patients with no residual viable invasive tumor cells in the lymph nodes will be classified as having an N-pCR. Existence of residual ductal carcinoma in situ (DCIS) or residual lobular carcinoma in situ (LCIS) is allowed in the diagnosis of pCR. When only invasive tumor was present at microscopic examination the response was graded as minimal residual disease. And when invasive tumor was visible at macroscopic evaluation of the mastectomy/lumpectomy specimen and axillary's lymph nodes, the pathological response was graded as macroscopic disease.

11. ADVERSE EVENTS

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) v3.0 for adverse event monitoring and reporting. The CTCAE v3.0 can be downloaded from the CTEP home page (http://ctep.info.nih.gov/reporting/ctc.html). All appropriate treatment areas should have access to a copy of the CTCAE v3.0.

11.1 Definition

11.1.1 Adverse events (AE)

An AE is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable or unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not it is related to the medicinal (investigational) product itself.

11.1.2 Serious adverse event (SAE)

An SAE is any adverse event that fulfils at least one of the following criteria:

- Results in death
- Is life-threatening (Note: the term "life-threatening" refers to an event in which the subject was at immediate risk of death at the time of the event; it does not refer to an event which could hypothetically have caused a death had it been more severe)
- Requires inpatient hospitalization or prolongation of existing hospitalization

- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect; or
- Requires medical intervention to prevent permanent impairment or damage.

11.1.3 Unexpected adverse events

An unexpected adverse event is an adverse event, the nature or severity of which is not consistent with the Investigator's brochure or summary of product characteristics.

11.1.4 Relationship to study medication

Relationship to study medication

Unrelated	This category is applicable to those AEs which are judged to be clearly and incontrovertibly due only to extraneous causes (disease, the environment, etc.)
Unlikely	This category is applicable to an AE that does not follow an anticipated response to the trial medication, which may be attributable to something other than the trial medication, and which is more likely to have been produced by the subject's clinical state or concomitant therapy.
Possible	This category is applicable to an AE that follows a reasonable temporal sequence from administration of the trial medication, that may be an anticipated response to the trial medication, but that could have been produced by the subject's clinical state or concomitant therapy.
Definite	The category is applicable to an AE that follows an anticipated response to the trial medication, and that is conformed by both improvement upon stopping the trial medication (de-challenge), and reappearance of the reaction on repeated exposure (re-challenge).

11.2 Reporting

11.2.1 Adverse event reporting

All adverse events spontaneously reported by the patient or observed by the Investigator must be recorded in the CRF. The obligation of recording the adverse event will be continued until one month after last course of neoadjuvant chemotherapy protocol treatment.

11.2.2 Serious adverse event reporting

Any serious adverse events must be reported to the Sponsor by telephone or facsimile (Tel: 02-23123456 ext.66048, Fax: 23813119, Ms. Jing-Fang LIN)

within 72 hrs following the discovery of the event. A written report should follow the oral report within 7 days to the Sponsor. The Sponsor will advise the Investigator of any further information or documentation that is required. The Investigator should also report all serious adverse events to the Ethics Committee/IRB (Institutional Review Board) and DOH (Department of Health in Taiwan) within the timeline according to their requirement. The obligation of reporting the severe adverse event will be continued until one month after last course of neoadjuvant chemotherapy protocol treatment.

11.3 Follow-up

Any adverse events that are still continuing at the time of one month after last course of protocol treatment final visit must be followed-up until resolution or stabilization, unless in the investigator's opinion, the condition is unlikely to resolve due to the patient's underlying disease.

12. STATISTICAL CONSIDERATIONS

12.1 Populations for analyses

The following data sets will be used in the study:
A. <u>Enrolled patients</u>: all patients who signed the informed consent and received an enrollment number assigned.
B. <u>Randomized patients</u>: all subjects who were randomized to one of the treatment arms.
C. <u>Treated patients</u>: all randomized subjects who received at least one dose of chemotherapy.

D. <u>Evaluable patients</u>: all patients who have received at least 3 cycles of chemotherapy and have received pathological response assessment unless treatment discontinue is due to disease progression.

All analyses will be performed using the treatment arm as randomized (unless otherwise specified) with the exception of dosing and safety, for which the treatment arm as received will be used.

12.2 Study endpoint definitions

12.2.1 pCR rate

The pCR rate in each arm will be defined as the number of patients in that arm who achieve a pathological complete response divided by the number of randomized patients in that arm.

12.2.2 **Objective clinical response rate**

The response rate (RR) in each arm will be defined as the number of patients in that arm who achieve a clinical response (PR or CR) divided by the number of randomized patients in that arm.

12.3 Statistical methods

12.3.1 Data analysis: The demographic and baseline measures will be summarized by treatment arm for all randomized patients using descriptive statistics. In addition, listings will be generated for all patients that were enrolled into the study but were never randomized. Statistical analyses will be performed using the SAS software (Version 9.1, SAS Institute Inc., Cary, NC, U.S.A.) and S-PLUS software (Version 7.0.4, Insightful Corporation, Seattle, WA, U.S.A.). Continuous data are expressed as mean ± standard deviation (SD) unless otherwise specified. Percentage is calculated for categorical variables. Student's *t* test and Wilcoxon rank-sum test are used to compare the means and medians of continuous data between two groups, whereas Chi-squared test and Fisher's exact test are used to analyze categorical proportions. In addition to univariate analyses, multivariate analyses of all potential prognostic factors are conducted by fitting multiple logistic regression model to predict patient's probability of pCR. Basic model-fitting techniques for (1) variable selection, (2) goodness-of-fit assessment, and (3) regression diagnostics (e.g., residual analysis, detection of influential cases, and check for mullkticollinearity) will be used in our regression analyses to ensure the quality of analysis results. Group sequential analysis is planned to allow 1 interim analysis (see below) before the end of the study with the overall alpha level of 0.05.

12.3.2 Safety analyses
   A. All safety analyses will be performed on all patients who are exposed to study medication, irrespective of whether the first cycle is completed or not.
   B. The safety analysis will report the frequency of all adverse events and laboratory abnormalities, as well as the frequency of dose interruptions, dose reductions and treatment discontinuation for toxicity in each treatment arm.
   C. The incidence of adverse events will be presented using the worst NCI-CTCAE grade per patient.

12.4 Interim analysis

A single interim analysis of the efficacy data will be conducted after 60% of evaluable patients, i.e., a total of 160 patients, have completed the study. The drift

parameter of the interim analysis has been calculated in the determination of required sample size using the Lan-DeMets alpha spending function method, which is available in the software developed by Michael A. Proschan, K.K. Gordan Lan, and Janet Turk Wittes (2006). The details of the calculation of sample size are stated in the next subsection..

12.5 Sample size determination

Under the assumption of pCR rate of 15% in TE arm, to achieve 80% power at the 5% alpha level of significance for the detection of a 15% increase of pCR rate in tailored regimen arm, 134 patients in either arm should be included in the study. To allow one interim analysis, we need a drift parameter of 2.8136, instead of 1.96 + 0.84 = 2.80 for a trial with no monitoring, using the O'Brien-Fleming boundary. Thus, the ratio of the sample size to achieve 80 percent power when monitoring with the Lan-DeMets alpha spending function and that with no monitoring is $(2.8136/2.80) \times 2 = 1.009$. That means the sample size must be 0.9 percent larger than a trial with no monitoring. Hence, the required sample size is $134 \times 1.009 = 136$ for each group in this sequential trial with two data analyses (including one interim analysis and the final analysis at the end of study). Based on the O'Brien-Fleming boundary, the alpha level spent in each analysis will be smaller than that in a trial with no monitoring. After considering a 10% drop-out rate and 5% multi-center study extra-variation, a total of 316 patients are required.

12.6 Independent Data Monitoring Committee (IDMC)

An Independent DATA Monitoring Committee (IDMC) will be set up for this study, which consists of the experts in clinical medicine, statistics, and medical ethics. The IDMC will meet on a regular basis to review the safety data, conduct the interim analysis, and handle ethical issues.. The first review will occur after the first 100 patients have finished at least 2 cycles of chemotherapy. A second review is scheduled to occur 12 months since the beginning of the trial. The interim analysis will be conducted in about 16 months after the beginning of the trial. Unscheduled and subsequent reviews will be decided on an ad hoc basis by the IDMC. The IDMC will also start provisional review within 1 month after the first SAE occur.

An independent statistician will provide the IDMC with relevant tables and listings. The safety data will include demographic data, AEs, SAEs and laboratory abnormalities (hematology and biochemistry). In addition, the IDMC will be provided with a copy of the SAE forms received. Further information will be given on request.

Following their data review, the IDMC will provide written recommendation as to whether the study may continue, possible amendment(s) required to the protocol, or whether the study should be stopped. The final decision will rest with the study principle investigators.

13. Correlative Translational Study

13.1 Rationale

Some potential biomarkers that are not target of chemotherapy have been reported to predict chemotherapy response. These markers include TS, P53, c-MYC and ATP-dependent transporter (ABCB1)[7,36,37]. In this study, we plan to validate the predictive power of these markers and their correlation with the 3 biomarkers used in this study. We will also try to explore other unknown predictive marker to predict chemotherapy response.

13.2 Additional biomarker study

Only for patients who have consented to participate in the correlative translation study.

13.2.1 Study method

Immunohistochemistry staining of TS, c-MYC, P53 and other potential predictive marker will be done in paraffin embedded blocks. Their expressions will be correlated with tumor response and other 3 biomarkers used in this study. We will also apply methods of cDNA (complementary DNA) microarray, and DNA polymorphism analysis to explore other unknown predictive markers.

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