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Pegylated liposomal doxorubicin as first-line monotherapy in elderly women with locally advanced or metastatic breast cancer: novel treatment predictive factors identified

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Abstract

We investigated the efficacy and safety of single-agent pegylated liposomal doxorubicin (PLD) as first-line treatment for elderly women with advanced breast cancer and evaluated predictive markers for response and toxicity.

25 women ≥ 65 years received 40 mg/m² PLD every 28 days. Time to treatment failure (TTF), response rate, time to progression (TTP), overall survival (OS), and analysis of the *ABCB1* single nucleotide polymorphism (SNP), the MRN complex, and TOPOII α were analyzed. A mean of 7.4 cycles PLD were administered and TTF was 5.5 months and OS 20.6 months.

ABCB1 SNPs were found to correlate to both efficacy and toxicity, while tumor expression of the MRN complex and TOPOII α correlated to TTP.

PLD is a safe and effective treatment for elderly breast cancer patients. Also potential predictive markers were identified.

Key words:

Anthracycline, elderly, genotype, metastatic breast cancer, MRN complex, topoisomerase II α

1. Introduction

A common clinical dilemma regarding elderly women with advanced breast cancer is the choice of chemotherapy, as approximately 50% of diagnoses occur after age 65 and few clinical trials have studied aged patients [1-4]. Treatment of elderly can

be challenging due to comorbidity, risk of toxicity, and reduced quality of life. For these reasons, single-agent therapy is generally advocated [5,6]. Evaluating different chemotherapy regimens for elderly women with breast cancer should include investigating factors that could facilitate selection of patients with the best chance of treatment benefit. Efficacy and toxicity are influenced by both host and tumor factors, which may include the expression of P-glycoprotein (encoded by the ABCB1 gene) and DNA repair proteins [7].

Anthracyclines are among the most active agents for the treatment of breast cancer, but are limited by toxicities including myelosuppression, mucositis, nausea and vomiting, and of special concern in the elderly, cardiotoxicity [2,8]. Pegylated liposomal doxorubicin (PLD) is a liposomal encapsulated form of doxorubicin with a longer half-life, higher tumor tissue penetration, and reduced toxicity [9]. A large phase III study demonstrated PLD to have similar efficacy, improved cardiac safety, and a lower incidence of alopecia, nausea, vomiting, and myelosuppression compared with conventional doxorubicin as first-line treatment of metastatic breast cancer (MBC) [9]. However, PLD was associated with a higher incidence of palmar-plantar erythrodysesthesia (PPE) and mucositis. A reduction of PLD from standard dose 50 mg/m² to 40 mg/m² resulted in comparable efficacy, but reduced incidence of PPE in a prospective phase II trial [8,18].

The aim of this study was to investigate the efficacy and safety of single-agent PLD 40 mg/m² as first-line treatment for elderly women with breast cancer, either locally-advanced or metastatic, and to search for predictive markers for treatment response and toxicity. These included single nucleotide polymorphisms (SNPs) of the

ABCB1 gene and tumor tissue investigations for the drug-related target topoisomerase II α (TOPOII α) and the MRE11/RAD50/NSB1 (MRN) complex involved in DNA repair [10].

2. Materials and Methods

2.1 Eligibility Criteria

Eligible patients included women ≥ 65 years with inoperable, locally advanced, or metastatic breast cancer with measurable or non-measurable disease. Patients had to demonstrate WHO performance status 0–2, a left ventricular ejection fraction (LVEF) $\geq 50\%$ with no clinical signs of heart disease, and normal organ function (except for patients with liver metastases, ALT and/or AST ≤ 5 times the upper normal limit was accepted). Adequate bone marrow function defined as platelets $\geq 100 \times 10^9/L$, neutrophils $\geq 1.5 \times 10^9/L$, white blood cells (WBC) $\geq 3.0 \times 10^9/L$, and a hemoglobin $> 90g/L$ was required as was a life expectancy of ≥ 12 weeks. All patients received oral and written information, and provided written informed consent.

Major exclusion criteria included previous chemotherapy for MBC, recurrence < 12 months after adjuvant anthracycline, myocardial infarction within 6 months of planned inclusion, and symptomatic brain metastasis. Patients eligible for trastuzumab or endocrine therapy were excluded.

The study was approved by the Ethics Committee at the University Hospital in Linköping, Sweden (M172-06), and the trial was registered at www.clinicaltrials.gov (NCT 0060 4968).

2.2 Study Design

This was a multicenter, open, single-arm, phase IV study. Patients received PLD 40 mg/m² intravenously every four weeks. Dose modifications were permitted for hematologic and non-hematologic toxicity. Treatment was continued until progression, unacceptable toxicity, or by physician or patient decision to withdraw from the study. Efficacy and safety were assessed.

2.3 Study Objectives

The primary endpoint of the study was time to treatment failure (TTF). Treatment failure was defined as progression of disease or unacceptable toxicity leading to discontinuation of treatment or death. Secondary endpoints were safety, response rate, time to progression (TTP), overall survival (OS), and analysis of potential treatment predictive factors, including SNPs of the ABCB1 gene and analyses of tumor tissue.

2.4 Efficacy Measurements

For all patients TTF, OS, number of patients requiring dose reduction, and total number of cycles received were calculated. For patients with measurable disease TTP and response rate (complete response [CR], partial response [PR], stable disease [SD], and progressive disease [PD]) was determined according to RECIST criteria except in patients with bone metastasis where WHO criteria were used [11,12]. Both RECIST and WHO criteria were used for patients with measurable disease and bone metastases.

Tumor evaluation was performed by radiology and physical examination at baseline and every 12 weeks after the start of treatment. Radiological procedures

included a computerized tomography (CT) or ultrasound of the abdomen, CT or x-ray of the thorax, bone scan and x-ray of hot spots.

2.5 Safety Measurements

All adverse events (AEs) were graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 [13]. Serious AEs (SAEs) were defined as any adverse drug or biologic experience occurring at any dose resulting in death, life-threatening AEs, persistent or significant disability/incapacity, or in-patient hospitalization.

Cardiac monitoring was by ultrasonic echocardiography (UCG) with calculation of LVEF, and was performed every 12 weeks for patients having previously received anthracyclines and every 6 months for anthracycline-naïve patients. Once patients received 12 cycles of PLD, UCG was performed every 12 weeks unless the cumulative dose of doxorubicin exceeded 550 mg/m², where UCG was performed every 8 weeks.

2.6 Tumor Markers Including TOPOII α and the MRN Complex

Information was collected on the primary tumor and metastases for estrogen and progesterone receptors, HER2 status and tumor grade. Ki67 was assessed with the mouse monoclonal antibody MiB-1 m7240 (Dako, Carpinteria, CA, USA). Expression of MRE11, RAD50, and NSB1 proteins (forming the MRN complex) and TOPOII α in the primary tumor was determined using immunohistochemistry.

Sections of 4–5 μm , cut from paraffin-embedded, formalin-fixed tumor tissue, were deparaffinized and rehydrated. Antigen retrieval was performed by heating in 10% Diva solution (Dako, Carpinteria, CA, USA) in a decloaking chamber (Digital decloaking chamber DC2002, Biocare medical, Concord, CA, USA). The settings were 125°C for 30 seconds and 90°C for 10 seconds. Slides were then cooled down to room temperature for 20 minutes. Serum-free protein block (Dako) was used for all antibodies and phosphate buffered saline/0.1% bovine serum albumin was used for washing and antibody dilution. The MRE11 (Clone 12D7) and RAD50 (13B3/2C6) antibodies (Abcam, Cambridge, UK) were used at 1:200 dilution for 1 hour at room temperature and at 1:400 dilution overnight at 4°C, respectively. The p95/NBS1 antibody (Cell Signaling Technology, Beverly, MA, USA) was used at 1:100 dilution for 2 hours at room temperature, and the TOPOII α (clone Ki-S1) antibody (Dako) was used at 1:100 dilution overnight at 4°C. DAKO EnVision+ was used as secondary antibody for 30 minutes at room temperature. The immunostaining was visualized using 3, 3'-diaminobenzidine and hydrogen peroxide for 8 minutes. After washing, the slides were counterstained with hematoxylin, dehydrated, and mounted.

2.7 Evaluation of Immunostaining

The immunohistochemical staining was evaluated independently by two investigators blinded to clinical data. In cases of discrepant scoring, a consensus score was reached after re-evaluation. For the MRN proteins, a proportion score, representing the estimated proportion of stained cell nuclei (0=no stained cell nuclei; 1=1–25%; 2=26–50%; 3=51–75%; 4=76–100%) and an intensity score, representing the intensity of the stained cell nuclei (0=no staining; 1=weak staining; 2=moderate staining;

3=strong staining) was assigned. For TOPOIIa, the proportion score was slightly different (0=<10%; 1=11–25%; 2=26–50%; 3=51–75%; 4=76–100%). The proportion and intensity scores were then added to obtain a total score, ranging from 0–7.

2.8 Genotyping of ABCB1

Genomic DNA was isolated from whole blood using the Maxwell 16 blood DNA purification kit from Promega (Madison, WI, USA). The genotypes for the ABCB1 SNPs G1199A, C1236T, G2677T/A, and C3435T were determined using pyrosequencing as previously described [14,15]. In short, a HotStarTaq master mixture (VWR International, Stockholm, Sweden) was used for PCR amplification, and all reactions were carried out on a Mastercycler gradient instrument (Eppendorf, Germany) in a total volume of 25 µL. The SNPs were analyzed using a Pyrosequencing PSQ96MA instrument (Qiagen, Stockholm, Sweden) according to the manufacturer's protocol by annealing a specific sequencing primer to a single stranded PCR-product and dispensing single nucleotides in a predesigned order [14,15].

2.9 Statistical Methods

The primary endpoint, median TTF, was assumed to be 7 months. A sample size of 25 patients was expected to produce a 95% confidence interval (CI) of 3.6–12 months. TTF was measured from date of first treatment to date of event and censored for patients with no event at the date of last visit. TTF, TTP, and OS were calculated by using the Kaplan-Meier estimate and log-rank test when comparing subgroups of patients.

Response and progression were defined as the first evaluation that showed response or progression, respectively. Response had to be confirmed by two observations not less than 4 weeks apart. Duration of response was defined as the time span between first response and first progression.

3. Results

3.1 Patient Demographics

Twenty-five patients were enrolled at five centers in Sweden from February 2007 to October 2008. Median patient age was 72 years (See table 1 for Patient and Tumor Characteristics).

3.2 Efficacy

Median TTF was 5.5 months (CI 95%: 3.67, 8.52), median TTP was 5.7 months (CI 95%: 3.74, 13.8), and median OS was 20.6 months (CI 95%: 6.58, 25.6). Of the 25 enrolled patients, three had non-measurable disease and were not included in the response analysis. They were withdrawn from the study without documented progression after 6, 7, and 11 cycles, respectively. Eighteen patients were assessed by RECIST or a combination of RECIST and WHO criteria, and four by WHO criteria only. Of these, three achieved a PR, and 13 had SD; six had PD as best response. Six of the 16 patients with SD or non-measurable disease received ≥ 7 cycles of therapy, resulting in 6 months or longer disease stabilization.

3.3 Safety

Patients received a median of 6 cycles and mean of 7.4 cycles (range 1–21 cycles) of therapy. The majority of AEs were mild to moderate in severity and transient; no grade 3–4 hematologic toxicities were noted. The main related toxicities (all grades) were nausea (60%), fatigue (56%), and PPE (52%) (Table 2— grade 3 and 4 toxicity). Four SAEs were reported possibly related to PLD.

Clinically significant cardiac events were noted in three patients: one grade 2 heart valve dysfunction and grade 1 left ventricular dysfunction; one grade 4 myocardial infarction and heart failure; and one grade 2 tachycardia, the latter two leading to PLD discontinuation. Four decreases in LVEF did not meet criteria for study withdrawal.

Study drug was also discontinued in two patients due to severe PPE. PLD dose was reduced in 10 patients, six due to weight loss and four due to: PPE(2), rash(1), and diarrhea(1).

3.4 Single Nucleotide Polymorphism in ABCB1 Gene

Twenty-four patients were genotyped for the ABCB1 SNPs (Table 3). One patient with a G/A genotype of G2677T/A was excluded from further analysis. For the variant G1199A, only 1 patient had the G/A genotype, and this SNP was not evaluated for clinical response. TTF by ABCB1 genotypes G2677T and C3435T are shown in Figure 1, and the genotype effects on TTP are shown in Figure 2. The mean TTF and mean TTP for the ABCB1 genotypes are presented in Table 3. The estimated mean TTF and TTP were significantly longer for patients who were wild type for G2677T, intermediate for heterozygous, and shortest for homozygous variant patients. A similar, but non-significant trend was seen for the ABCB1 SNP C3435T. Patients with the 3435T/T

genotype were significantly more susceptible to moderate to severe PPE ($P=0.02$), where 5 of the 8 T/T-patients suffered moderate to severe PPE, while this was true for only 2 of 16 patients with non-3435T/T genotype (Figure 3). No correlation between myelosuppression or cardiotoxicity and ABCB1 genotype was demonstrated.

3.5 Investigations of Primary Tumor Tissue

No significant association of grade, estrogen or progesterone receptor and HER2 status, with TTF or TTP was found. For seven patients with triple-negative tumors TTF and TTP were similar to those in the other subgroups. Patients with tumors with low proliferation rate (Ki67 $\leq 10\%$) had significantly longer TTF ($P=0.00051$) and TTP ($P=0.0062$) than those with intermediate or highly proliferative tumors (Figure 4A). (See table 1 for Patient and Tumor characteristics) Patients with a high intensity score for tumoral TOP2 α showed non-significant trends of longer TTF ($P=0.19$) and TTP ($P=0.20$). The TOP2 α score correlated with Ki67 ($P=0.044$). To adjust for this association, a ratio between the TOP2 α intensity and Ki67 scores was established and patients were categorized into two groups with a low ratio ($n=10$) and a high ratio ($n=10$), respectively. Patients with a high ratio showed significantly longer TTF ($P=0.0028$) and TTP ($P=0.0043$) than patients with a low ratio (Figure 4B).

The immunoscores for the individual proteins in the MRN complex were not significantly associated with TTF or TTP, although a low score (<6) for RAD50 ($P=0.096$) as well as a low score for MRE11 ($P=0.20$) was weakly associated with longer TTP. An MRN scoring system was created such that a low score was defined by all three proteins individually having low scores (<6). None of the six patients with a low

MRN score showed progression during the time of follow-up (Figure 4C). Figure 4D-F show examples of the immunostaining for Ki67, TOP2A and MRE11

The findings regarding the MRN score and TOP2A were largely independent of other prognostic tumor characteristics, such as grade and hormone receptor status (data not shown).

4. Discussion

The optimal treatment for women over 65 years with locally advanced or MBC has not yet been determined. Exploring the role of different chemotherapeutic agents in this setting via clinical trials is necessary for evidence-based treatment recommendations. Identifying predictive markers for chemotherapy is becoming increasingly important to optimize treatment efficacy and diminish patient suffering due to toxicity.

We conducted a phase IV trial in 25 women with locally advanced or MBC, not amenable to curative treatment, investigating the efficacy and safety of PLD in the first-line setting at a dose of 40 mg/m² every 28 days until treatment failure. We explored the role of TOP2A expression and the MRN complex in tumor tissue and different SNPs of the ABCB1 gene in relation to efficacy and treatment toxicity. Despite the small size of this trial, we found clinically interesting correlations.

O'Brien et al compared PLD 50 mg/m² every 4 weeks with doxorubicin 60 mg/m² every 3 weeks as first-line treatment for MBC [9]. Median age was 58 years (range 25–82), with 28% being ≥65 years. Efficacy was similar between treatment arms, with significantly less cardiac events in the PLD arm. Response rate for PLD was 33%, and OS was 21 months. PPE grade 3/4 was noted in 17% and stomatitis in 9% of patients.

Similarly, the EORTC reported a retrospective joint analysis of two trials (including data from the study of Coleman) utilizing two different dosing schedules of PLD conducted mostly in patients >65 years [16,17]. PLD schedules were 50 mg/m² every 4 weeks or 60 mg/m² every 6 weeks. Tolerability and efficacy in patients <70 years versus ≥70 years were compared, with equivalent efficacy demonstrated (ORRs of approximately 30%). Toxicity was higher in the older cohort for the six-week schedule, but also noted in the four-week schedule were more treatment discontinuations for toxicity (mainly skin) among elderly patients versus younger patients (29% vs 14%, respectively). Al-Batran et al (8,18) studied response and toxicity of PLD in two different cohorts of pretreated breast cancer patients, the first group receiving 50 mg/m² and the second 40 mg/m². For both studies ORR was 13%, while PPE grade 3/4 was reduced from 6 to 0% and stomatitis grade 3/4 from 10 to 4% by decreasing the dose.

In our study, 3 of 22 patients with measurable disease (14%) had a PR, no patients had a CR, and 13 of 22 patients (60%) achieved SD. Only 4 patients (16%) discontinued treatment because of PLD-related AEs: 2 with severe PPE and 2 with cardiac events. Toxicity data compare favorably to the Coleman and O'Brien results of 50 mg/m² PLD although PPE was more frequent than among the mainly younger patients in the 40 mg/m² PLD study of Al-Batran. (8,9,16,17,18).

Despite few objective responses, OS of 20.6 months was comparable to that observed in the O'Brien study. TTP was 5.7 months, which is also similar to the other first line studies (range 5.4-6.9 months) using higher dosing (Table 4).

The use of single-agent chemotherapy in our study facilitated identifying important biomarkers that may correlate to outcome or AEs. In the ABCB1 genotype analysis, the

G2677T genotype had a significant impact on TTF and TTP and C3435T had a non-significant trend on TTF and TTP (Figures 1 and 2). TTF and TTP were longest for patients with the wild-type genotype, intermediate for those being heterozygous, and shortest for homozygous variant patients. The G2677T genetic variation results in an amino-acid change from an alanine residue to a serine in P-glycoprotein at position 893, while the C3435T SNP is silent. Due to the fact that the G2677T and C3435T SNPs are linked, our findings corroborate results from Cizmarikova et al [19] who found that patients with the 3435C/C genotype had a longer TTP and an enhanced therapeutic outcome after neoadjuvant anthracycline-based chemotherapy. In accordance, Turgut et al [20] showed a trend toward a shorter PFS for breast cancer patients with 3435T/T compared to heterozygous and wild-type patients. They found no correlation between ABCB1 genotype and response, as also demonstrated by Rodrigues et al [21].

Patients with the ABCB1 C3435T/T genotype were significantly more susceptible to moderate to severe PPE affecting 62% of patients compared to 12% of those with non-3435T/T genotype (Figure 3). PPE is believed to be due to PLD excretion through the skin; therefore, an enhanced efflux might promote this toxicity [22]. No correlation between myelosuppression or cardiotoxicity and ABCB1 was demonstrated.

Retrospective studies have shown that amplification of the gene encoding *TOP2A* predicts sensitivity to anthracyclins [23-25]. As *TOP2A* amplification is almost exclusively found in tumors with HER2 amplification, and a positive HER2 status was an exclusion criterion in our study, we could not justify performing *TOP2A* analysis with FISH. However, sensitivity to anthracycline-based therapy has also been

related to high *TOP2A* expression levels and we hypothesized that high TOPOII α levels, also in the absence of gene amplification, might be predictive [26].

Immunohistochemical staining of TOPOII α likely reflects abnormal expression of the protein, DNA replication, or both, and a positive correlation between TOPOII α and Ki67 was observed. Accordingly, the TOPOII α :Ki67 ratio more accurately predicted TTP than TOPOII α alone.

The MRN complex is an active component in DNA double-strand break repair and may therefore play a role in chemotherapy effect. For cancer cells to resist treatment with drugs that fix topoisomerases to DNA, the topoisomerase must be released. Interestingly, MRN has been shown to be involved in topoisomerase removal, suggesting that impaired MRN function may increase the half lives of DNA-topoisomerase complexes, leading to higher treatment efficiency [27,28]. This might be a mechanistic explanation of our finding that patients with reduced MRN expression were less likely to progress.

In summary, our study demonstrated that PLD 40 mg/m² is a safe and effective treatment for elderly women with breast cancer. We found that SNPs of the ABCB1 gene correlate to both TTF and TTP and the risk of PPE, thereby indicating that the ABCB1 genotype could be a predictive factor for therapy with PLD in these patients. We also showed that expression of TOPOII α and the MRN complex influenced treatment outcome.

Additional investigations of the SNPs of ABCB1, gene expression of TOPOII α and the MRN complex in different age groups receiving PLD or other anthracyclines will help to further define their role for individualized treatment recommendations.

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Conflict of Interest Statement

The following authors of this paper declare that there is no conflict of interest involved in this paper: HG, OS, KB, ML, LC, JH, BN, AM. The following authors declare a conflict of interest: At the time of the study LMB was Project Physician for the study while being employed by Schering-Plough as a Medical Affairs Manager. ÅF previously held a position as Clinical Project Manager at Schering-Plough Sweden and now holds the same position at MSD Sweden. AA currently holds a position as Medical Affairs Manager (Medical Advisor) in oncology at MSD Sweden.

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Table 1. Patient and Tumor Characteristics

Patient Characteristics	N=25
Mean age, years (range)	72.3 (65-81)
WHO PS	
0	10 (40%)
1	13 (52%)
2	2 (8%)
Previous adjuvant anthracycline	6 (24 %)
Mean baseline LVEF (range)	61.3% (50-75%)
Tumor Characteristics	
ER status	
Positive	18 (72%)
Negative	7 (28%)
PR status	
Positive	9 (36%)
Negative	16 (64%)
HER2 status	
Positive	1 (4%) [†]
Negative	24 (96%)
Triple-negative	
No	18 (72%)
Yes	7 (28%)
Tumor grade [‡]	
1	2 (9%)
2	8 (35%)
3	13 (56%)
Ki67	
≤ 10%	6 (30%)
11-20%	5 (25%)
> 20%	9 (45%)

[†]Positive HER2 status was determined for metastatic tumor; HER2 status of primary tumor was not assessed. [‡]Grading available for 23 patients.

Table 2. Grade 3-4 Treatment-Related Non-Hematologic Adverse Events

	Grade 3	Grade 4
Diarrhea	1 (4%)	0
Nausea	1 (4%)	0
Vomiting	1 (4%)	0
Fatigue	1 (4%)	0
PPE	2 (8%)	0
Rash	1 (4%)	0
Infection	1 (4%)	0

Table 3. Mean TTF and TTP by Single Nucleotide Polymorphism in ABCB1 Gene

SNP genotype	Mean TTF Days (95% CI)			Mean TTP Days (95% CI)		
	C1236T	G2677T	C3435T	C1236T	G2677T	C3435T
Wild-type patients	n=11	n=9	n=4	n=11	n=9	n=4
	252 (130-374)	270 (140-401)	363 (83-634)	378 (228-527)	414 (264-565)	470 (228-712)
Heterozygous patients	n=10	n=12	n=12	n=10	n=12	n=12
	203 (114-291)	256 (129-385)	235 (137-382)	235 (137-334)	298 (165-431)	301 (185-417)
Homozygous variant patients	n=3	n=2	n=8	n=3	n=2	n=8
	266 (0-614)	88 (84-92)	143 (84-204)	266 (0-550)	88 (84-92)	180 (105-255)

Table 4. Comparative Trials

Study	Number of patients	Line of treatment metastatic disease	Dose PLD	Phase	Age median (range)	OR (CR+PR) %	SD %	TTP months	OS months	Cardiac tox % all grades	PPE % grade 3/4	Stomatitis+ mucositis % grade 3/4
O'Brien [9]	254 ^a	1 st line	50 mg/m ² q 4w	III ^b	59 (28-82)	33	25	6.9	21	5	17	9
Al-Batran [18]	79 ^c	≥ 2 nd line	50 mg/m ² q 4w	II	58 (35-79)	13	28	3.6	12.3	0 ^d	6	10
Al-Batran [8]	46	2-3 rd line	40 mg/m ² q 4w	II	60 (35-82)	13	35	3.3	10.7	0	0	4
Coleman [17]	116	1 st line 51/59 pts ^e	50 mg/m ² q 4w	II ^f	69 (43-85)	31	33	5.4	not stated	2 Grade 3/4	16	14
		1 st line 49 /57 pts ^e	vs 60 mg/m ² q 6w	II ^f	69 (37-87)	29	43	5.8	not stated	0 Grade 3/4	2	35
This study	25	1 st line	40 mg/m ² q 4w	II	72 (65-81)	14	60	5.7	20.6	12	8	0

^aOf the 509 patients in the trial, 254 were randomized to PLD

^bCompared to doxorubicin 60 mg/m² q 3w

^cAnthracyclin pretreated patients

^d5% of patients had ECG changes related to disease

^eRemaining patients treated ≥ 2nd line

^fRandomized phase II study

Figure 1.

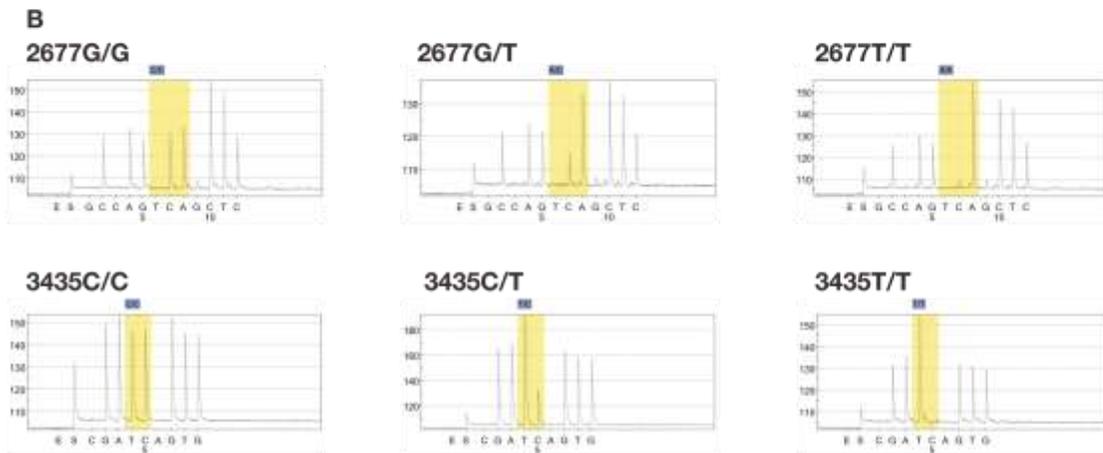
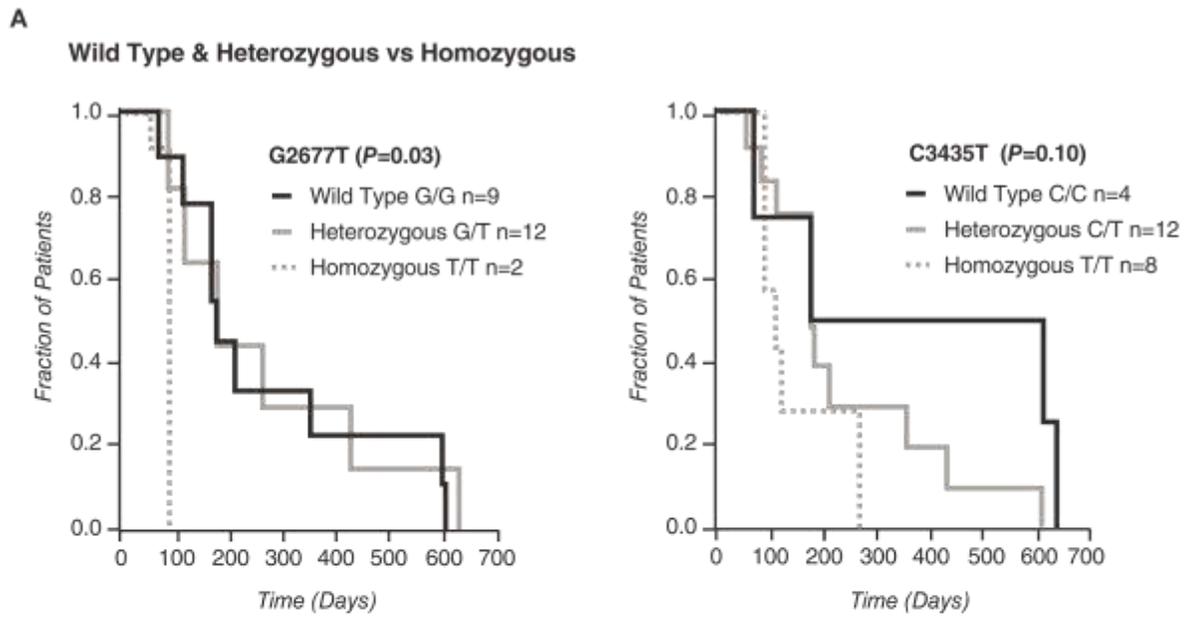


Figure 2.

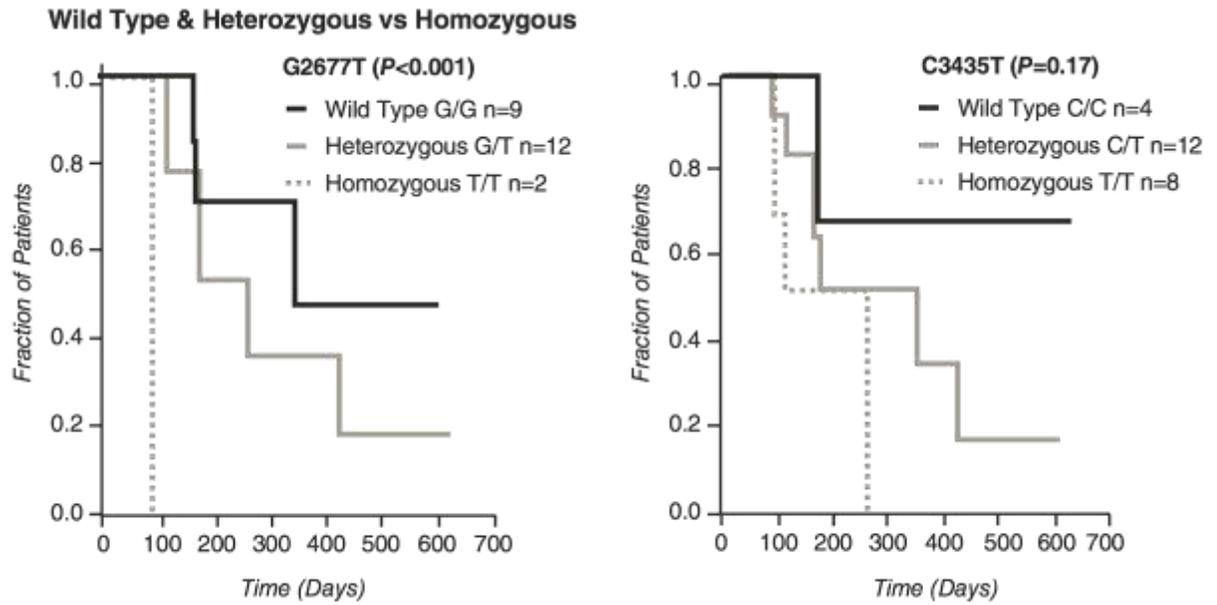


Figure 3.

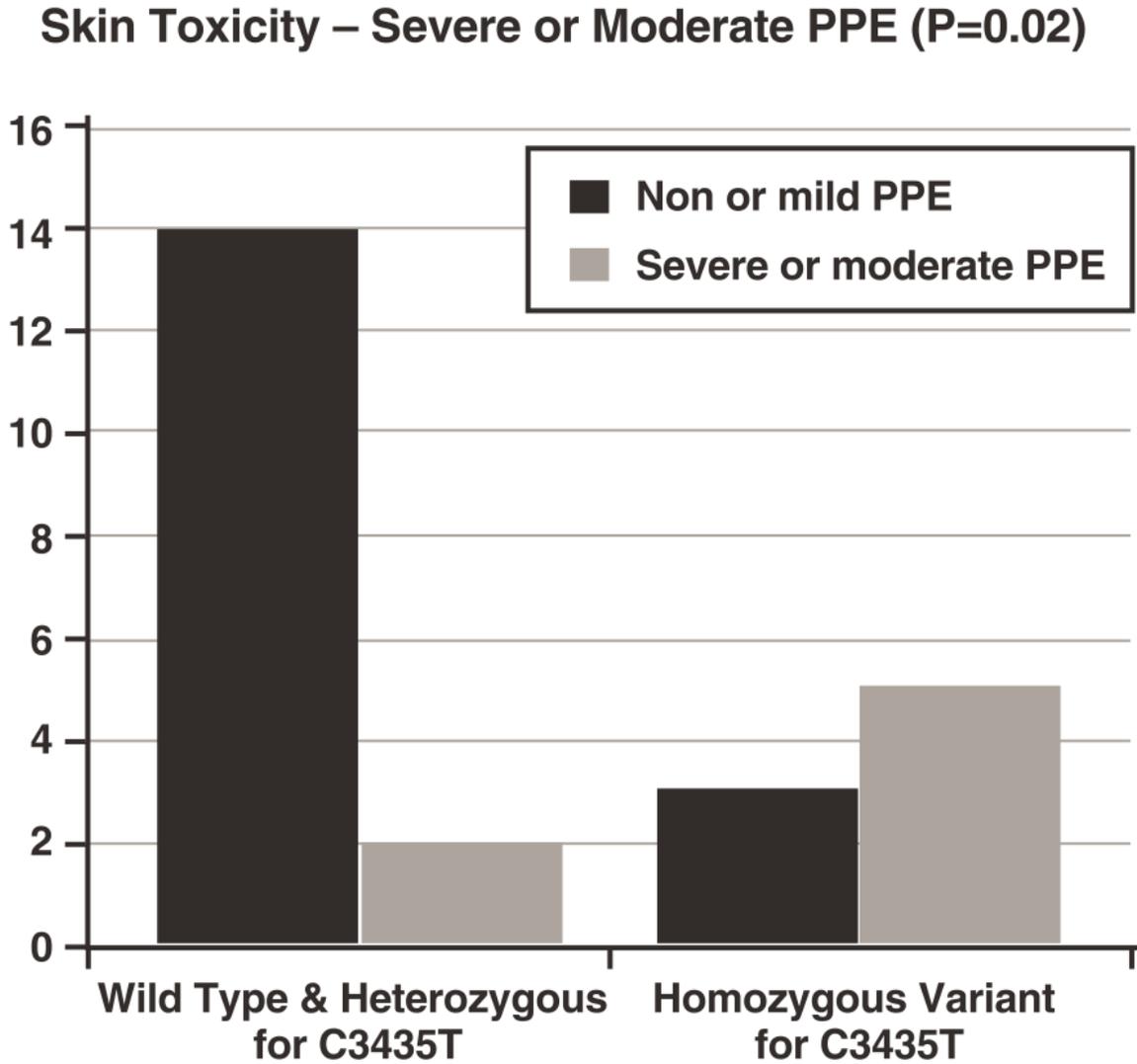


Figure 4.

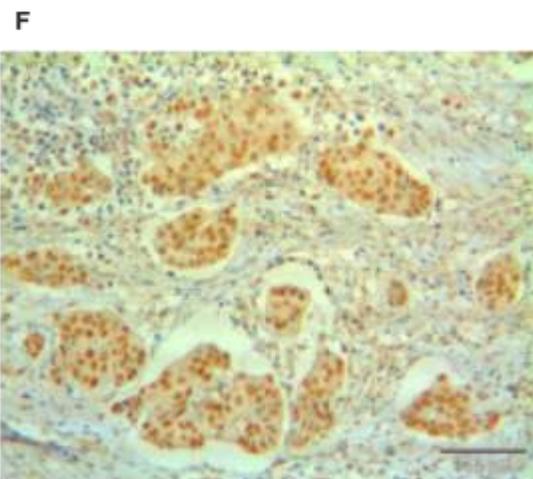
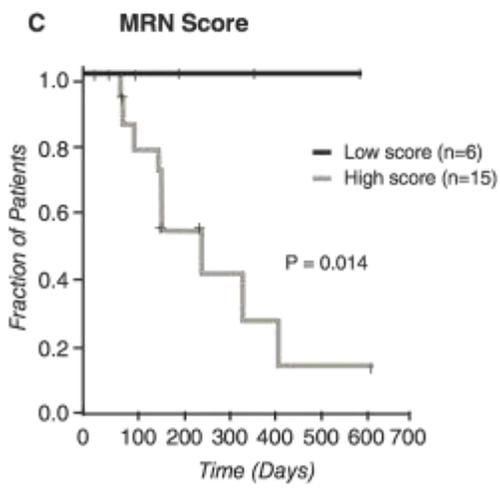
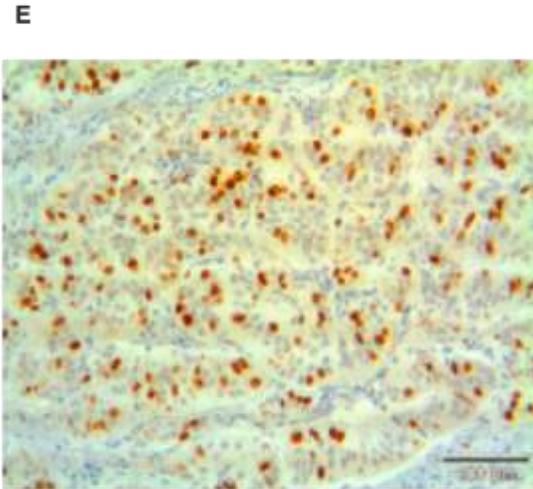
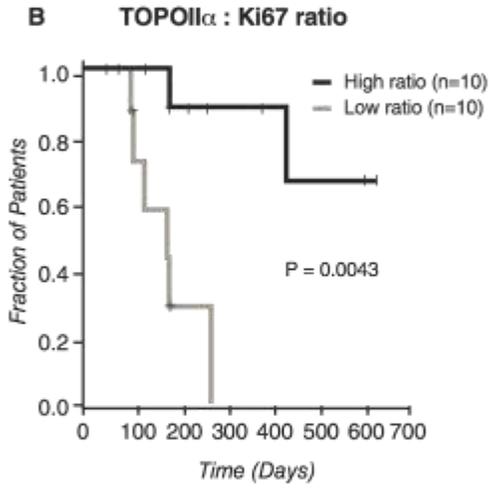
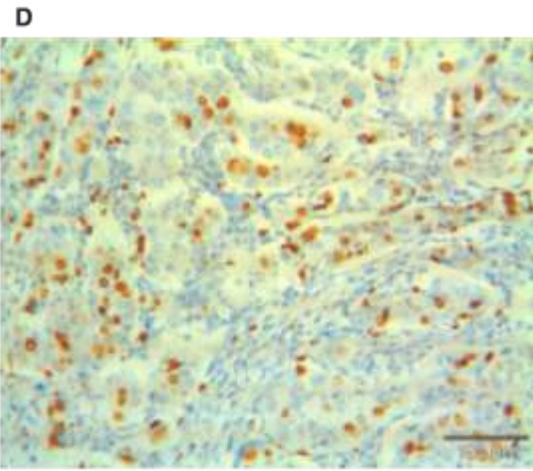
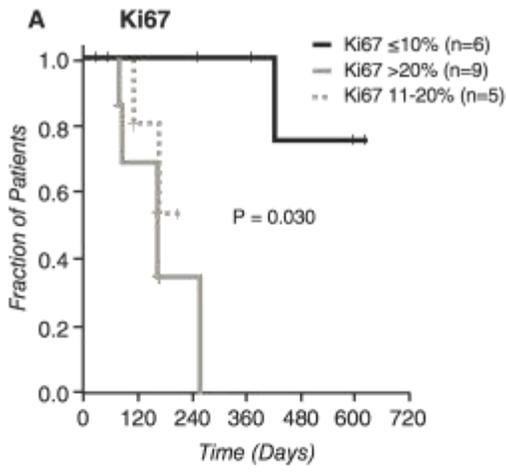


Figure Captions

Fig. 1 A) Effects of ABCB1 genotypes G2677T and C3435T on time to treatment failure. Patients homozygous (T/T) for *ABCB1* SNP G2677T had a significantly shorter TTF when compared with patients having wild type (G/G) and heterozygous genotypes (G/T) $P=0.03$.

B) The pyrograms for the sequencing of the SNPs G2677T and C3435T. The G2677T SNP is sequenced on the reverse strand and the upper part shows the pyrograms for 2677G/G, 2677G/T and 2677T/T. C3435T is sequenced on the forward strand and the lower part shows the pyrograms for 3435C/C, 3435C/T and 3435T/T.

Fig. 2 Effects of ABCB1 genotypes G2677T and C3435T on time to progression. Patients homozygous (T/T) for *ABCB1* SNP G2677T had a significantly shorter TTP when compared to patients having wild type (G/G) and heterozygous genotypes (G/T) $P<0.001$.

Fig. 3 Correlations between ABCB1 genotype and toxicity. Patients homozygous variant (T/T) for *ABCB1* SNP C3435T were more susceptible to palmar-plantar-erythrodysesthesia compared to wild type (C/C) and heterozygous (C/T) patients $P=0.02$.

Fig. 4 Time to progression in relation to Ki67 (A), TOPOII α :Ki67 ratio (B), and MRN score (C). Ki67 could be scored for 20 of the patients. TOPOII α and MRN score were

possible to assess for 21 patients. Tumor tissue from one of the patients immunostained for Ki67, scored as high (D), TOP2A, strong intensity and high TOP2A:Ki67 ratio (E), and MRE11, high score, representing one of the proteins in the MRN complex (F).

Footnote: abbreviations:

AE: adverse event

CR: complete response

CT: computerized Tomography

LVEF: left ventricular ejection fraction

MBC: metastatic breast cancer

MRN complex: MRE11/RAD50/NSB1

OR: overall response

OS: overall survival

PD: progressive disease

PLD: pegylated liposomal doxorubicin

PPE: palmar-plantar-erythrodysesthesia

PR: partial response

PS: performance status

SAE: serious adverse event

SD: stable disease

SNP: single nucleotide polymorphism

TOPOII α : topoisomerase II α

TTF: time to treatment failure

TTP: time to progression

UCG: ultrasonic echocardiography