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# Effectiveness of tocotrienol-rich fraction combined with tamoxifen in the management of women with early breast cancer: a pilot clinical trial

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## Abstract

**Introduction:** Basic research has indicated that tocotrienols have potent antiproliferative and proapoptotic effects that would be expected to reduce the effect of breast cancer.

**Methods:** We conducted a double-blinded, placebo-controlled pilot trial to test the effectiveness of adjuvant tocotrienol therapy in combination with tamoxifen for five years in women with early breast cancer. Two-hundred-forty women, aged between 40-60 years, with either tumor node metastases (TNM) Stage I or II breast cancer and estrogen receptor (ER) positive tumors were non-randomly assigned to two groups. The intervention group received tocotrienol rich fraction (TRF) plus tamoxifen whilst the control group received placebo plus tamoxifen, for five years.

**Results:** During the five years of study, 8 patients died due to breast cancer while 36 patients developed local or systemic recurrence. Five-year breast cancer specific survival was 98.3% (95% confidence interval (CI): 95.9% to 100%) in the intervention group and 95%, (95% CI: 91.1% to 98.9%) in the control group, while 5-years disease free survival was 86.7% (95% CI: 80.6% to 92.8%) and 83.3% (95% CI: 76.6% to 90.0%), respectively. Risk of mortality due to breast cancer was 60% (HR: 0.40; 95% CI: 0.08 to 2.05) lower in the intervention group versus the controls following adjustment for age, ethnicity, stage and lymph node status but this was not statistically significant. Adjuvant TRF therapy was not associated with breast cancer recurrence (HR: 0.84; 95% CI: 0.43-1.65).

**Conclusions:** From the current study, there seems to be no association between adjuvant

tocotrienol therapy and breast cancer specific survival in women with early breast cancer.

**Trial registration:** ClinicalTrials.gov Identifier: NCT01157026.

## Introduction

Vitamin E is a potent antioxidant which is classified into subgroups of tocopherols and tocotrienols. In most food sources, tocopherols are more prevalent than tocotrienols. Palm oil is a particularly rich source of α-, γ-, δ –tocotrienol [1,2]. Previous studies have reported that individual tocopherols and tocotrienols exhibit different potencies in suppressing tumor cell growth and inducing apoptosis in neoplastic mammary epithelial cells [3,4]. Administrations of α- and γ-tocotrienol, and not α-tocopherol, have shown a life prolonging effect in mice from transplanted tumors [5]. Tocotrienols were also found to suppress the growth of human breast and colorectal cancer cells [6–8] but not α-tocopherol. The above effects of tocotrienols may be explained through mechanisms such as antiangiogenesis, antiproliferation, induction of apoptosis and improving immunological functions [9].

Since tocotrienols are more potent than tocopherols [10,11], we hypothesize that tocotrienol intake may be associated with improved survival and lower recurrence in patients with early breast cancer. We investigated the effectiveness of TRF in combination with tamoxifen compared to placebo with tamoxifen as adjuvant therapy in improving breast cancer specific survival in women with estrogen receptor (ER) positive tumors who had been treated for TNM stage 1 and stage 2 (early) breast cancer. It should also be noted that tocotrienols with tamoxifen

have showed a synergistic inhibitory effect on the proliferative rate and growth of breast cancer cells *in vitro* [12].

## Materials and methods

### Study Design

We conducted a double-blinded, placebo controlled trial comparing tocotrienol-rich fraction (TRF) plus tamoxifen against placebo plus tamoxifen in women with early breast cancer for five years. A total of 240 women with early breast cancer were assigned into 2 treatment groups by non-random allocation. The intervention group was given TRF plus tamoxifen, (n = 120) while control group was given placebo plus tamoxifen, (n = 120). The primary end point was breast cancer specific survival, defined as the time from allocation to treatment group to death due to breast cancer. Breast cancer specific survival was chosen as primary outcome based on overall health protective effects conferred by the multiple functions of tocotrienols including anti-cancer, anti-inflammatory and anti-oxidant effects. The secondary end points included recurrence (either local or systemic), biochemical parameters, liver function and plasma levels of vitamin E.

### Study Population

A total of 240 patients treated in Kuala Lumpur Hospital, Malaysia were recruited between November 2001 and November 2002. Only women with histologically confirmed primary breast cancer and estrogen receptor positive tumors (determined by immunohistochemistry test with a 10% cut-off point for positivity) were recruited into the study. Other criteria for eligibility included age of 40-60 years at the start of the tamoxifen therapy, and have; an Eastern Cooperative Oncology Group performance status of 0,1, or 2 (scored on a scale of 0 to 5, with lower scores indicating better function) [13]. Criteria for exclusion were the concurrent use of investigational drugs and estrogen receptor status negative or unknown.

**Treatment Program**

Patients were blinded to the treatment that they received. Both the TRF and placebo drugs were prepared and supplied by Hovid Sdn. Bhd., Malaysia. The TRF drug was prepared in a capsule form at a dose of 200mg per capsule. The placebo drug which contained soy oil without tocotrienols had similar appearance and taste as the TRF drug. Patients were allocated to two treatment groups that received a daily dose of two capsules (one active TRF 200mg capsule or placebo capsule, along with tamoxifen 20mg tablet). Patients were instructed to take the two capsules together at approximately the same time each day. Trial treatment continued for five years from the date of recruitment. Patients were assessed every three months for compliance by collection of bottles containing capsules and random visits to their homes by the research staff.

The assessors were also blinded to the treatment received by the trial patients. Baseline assessment included demographic and clinical profile such as age, race, breast cancer stage and involvement of lymph nodes. Clinical evaluations, routine blood test for hematology and blood chemistry assessment were performed every six months during year one and annually thereafter. Patients were monitored for compliance to follow-up, whereby those who defaulted will be contacted and when necessary, new appointment dates will be given. Patients underwent several biochemical screening tests such as complete blood count (CBC) and differential count, liver function tests. These biochemical parameters were also used to determine the health conditions of the patients. Mammography was performed annually throughout the study. Both local and systemic recurrence of disease was defined pathologically or on the basis of clinical or radiologic findings, and recurrences were dated at the time they were first detected. Additionally, all deaths recorded in this study were confirmed as due to breast cancer. The ethical clearance for this

study was approved by the Research and Ethics Committee of the Ministry of Health, Malaysia (National Medical Research Register: Research Registration ID: 5399 S1). Written informed consent was obtained from all patients.

**Vitamin E Extraction from Blood Plasma and HPLC Analysis**

Blood samples collected in heparinized tube were spun at 2000 rpm for 10 minutes at room temperature. The plasma was isolated from the sedimented red blood cells and transferred into a sterile 1.5 mL centrifuge tube. Following this, 500 μL of the plasma was then added to a tube containing 0.5 mL of 0.5% NaCl, and ethanol. Then, 400 μL of hexane was added into this tube. The mixture was shaken vigorously for an hour using a mini shaker. The tubes were then spun at 3000 rpm for 10 minutes at room temperature. After centrifugation, the clear hexane phase was transferred carefully into a clean vial and blow-dried under nitrogen gas. An aliquot of the lipid sample was reconstituted in 500 μL hexane. Then 10 μL of the sample and a standard solution mixture of α-tocopherols, α-, γ- and δ-tocotrienols was also injected accordingly into a HPLC system. The excitation wavelength and emission wavelength of the fluorescence detector were set at 295 and 325 ηm, respectively. The mobile phase was hexane-isopropyl alcohol (99.5/0.5, v/v) with a flow rate of 2 mL/min. The peak areas of the components in the sample were compared with those of the standards and were used for quantitative calculation.

**Statistic Analysis**

The sample size was calculated under the assumption of a five year disease specific survival of 95 percent in the tamoxifen plus placebo group and the detection of a difference of 5 percent in

the five year disease specific survival rate. These assumptions necessitated the enrollment of 240 women.

Categorical variables were described in proportion and compared using Chi-square test while continuous variables were described in means and compared using t-test. The analysis was based on intention to treat principle. Kaplan-Meier analysis was used to estimate breast cancer specific survival and disease free survival comparing treatment versus the control group. Survival curves were compared using log-rank test. Cox regression analysis was used to compute crude hazard ratios to determine the association between TRF plus tamoxifen intake and breast cancer specific death as well as recurrence of breast cancer, compared to the intake of placebo plus tamoxifen which is the reference group. This model was subsequently adjusted for age (continuous), TNM stage (1 or 2), ethnicity (Malay, Chinese, Indian) and lymph node involvement (yes, no). *P* values of $< 0.05$, 95% confidence interval (CI) of not including 1.00 for HR were considered as statistically significant. Data obtained during the study were processed using SPSS for Windows (Version 18.0; SPSS Inc., Chicago, IL).

## Results

### Patient Characteristics

The median duration of follow-up was 60 months, with complete follow-up of all patients who complied to their treatment throughout the study. Eight patients (2 in intervention group versus 6 in control group) had died due to breast cancer and 36 patients (16 in intervention group versus 20 in control group) had developed local or systemic recurrence. Figure 1 shows the CONSORT diagram of the study. The groups were well balanced with respect to demographic and tumor characteristics (Table 1). Approximately, 61% of patients in the TRF plus tamoxifen group and 49% of patients in the placebo plus tamoxifen group were in TNM stage 1 of breast cancer, while 39% of patients in the TRF + tamoxifen group and 51% in the tamoxifen group were in TNM stage 2 (p=0.07). A total of 58% of patients in the TRF + tamoxifen group and 63% of patients in the placebo plus tamoxifen group showed involvement of lymph node (p=0.51). The average number of lymph nodes involved among these patients was 1.5 and 1.1 respectively (p=0.15).

Based on Kaplan-Meier analysis, 5 year breast cancer specific survival in the TRF plus tamoxifen group was 98.3% (95%CI: 95.9% to 100%) compared to 95%, (95%CI: 91.1% to 98.9%) in the placebo plus tamoxifen group (Figure 2). P for log-rank test was 0.15. Whereas, the 5 year recurrence free survival was 86.7% (95%CI: 80.6% to 92.8%) and 83.3% (95%CI: 76.6% to 90.0%), respectively. P for log-rank test was 0.47.

The crude hazard ratio for breast cancer specific death in the TRF plus tamoxifen group compared to placebo plus tamoxifen group was 0.33 (95% CI: 0.07 to 1.61) (Table 2). Following adjustment for age, ethnicity, stage and lymph node status, the multivariate HR was 0.40 (95%CI: 0.08 to

2.05) and statistically not significant. Compared to placebo intake, intake of tocotrienol by patients with stage 1 or stage 2 breast cancer, and estrogen receptor positive tumor treated with tamoxifen, is associated with a non-statistically significant (p=0.27) 60% lower risk of mortality, following adjustment for age, ethnicity, stage and lymph node status.

Multivariate hazard ratio for local or systemic recurrence in the TRF plus tamoxifen group compared to placebo plus tamoxifen group was 0.84 (95% CI: 0.43-1.65).

**Biochemical parameters in study population**

The mean values of the results from the biochemical screening tests on day 0, year 1, 2, 3, 4 and 5 of TRF plus tamoxifen supplemented group and placebo plus tamoxifen supplemented group are shown in Table 3.

The biochemical parameters measured did not exhibit any significant changes from the results obtained before the start of the study for both TRF plus tamoxifen and placebo plus tamoxifen groups.

**Liver Function Tests**

The purpose of liver function tests is to study the tolerance of the supplement. The mean values of the results from the liver function tests on day 0, year 1, 2, 3, 4 and 5 of TRF plus tamoxifen supplemented group and placebo plus tamoxifen supplemented group are shown in Table 4.

Liver function tests (total protein, albumin and total bilirubin) showed insignificant values for prior to the start and upon completion of the study for TRF plus tamoxifen supplemented group and placebo plus tamoxifen supplemented group. Liver function tests such as alkaline phosphatase and alanine transaminase showed high standard deviation (SD) values. This is due to the collection of samples from patients after the chemotherapy session.

**High levels of Vitamin E in plasma of patients supplemented with TRF**

Plasma samples obtained from patients were analysed using HPLC to quantify the concentrations of vitamin E in plasma (Figure 3).

The amount of endogenous α-tocopherol in the blood of patients from both groups increased significantly ($P < 0.05$) after the five year period compared to day 0 (Figure 3). The α-tocopherol, α-, γ- and δ-tocotrienol concentrations increased significantly ($P < 0.05$) in patients who received TRF plus tamoxifen as compared to placebo plus tamoxifen (Figure 3).

However the concentrations of tocotrienols in the placebo group after the five year period remained the same and the amounts did not differ significantly ($P > 0.05$) as compared to day 0.

## Discussion

In our current pilot study, we found no protective association between tocotrienol intake and breast cancer related mortality or recurrence in patients with early breast cancer. Tamoxifen

alone and TRF plus tamoxifen supplementation also did not change the blood parameters of patients prior to the start and upon completion of the study.

The risk of dying due to breast cancer seems to be reduced by approximately 60% in patients receiving a combination of tocotrienol and tamoxifen compared to patients receiving placebo and tamoxifen. However this was not statistically significant ($p=0.27$). Previous studies carried out in our laboratory showed that tocotrienols inhibited growth and proliferation of breast cancer cells [6-8]. Another study reported that the anticarcinogenic property of tocotrienols in synergy with tamoxifen is more potent than tamoxifen alone [12]. Recently we have also unfolded a novel mechanism by which tocotrienols inhibit breast cancer cell growth where the effects are mediated by binding to the ERβ and inducing the expression of specific genes containing Estrogen Responsive Element (ERE) sequences in their promoter [14].

Our results showed that TRF plus tamoxifen supplemented group had significantly higher concentrations of α-tocopherol and α-, γ- and δ-tocotrienols in the plasma of the breast cancer patients. Plasma α-tocopherol levels were much higher than any other tocotrienol isomers. This result supports the findings reported by [15], who reasoned that α-tocopherol transfer protein (α-TTP), together with the tocopherol associated proteins (TAP) are responsible for the endogenous accumulation of natural α-tocopherol.

Concentrations of the micronutrient in blood plasma revealed that α- tocotrienols had the highest absorption, followed by γ- and δ-tocotrienols in all individuals supplemented with tamoxifen and

TRF at the end of study as compared to placebo and basal concentrations of the micronutrients. Although the α-TTP which binds the vitamin E isoforms via LDL have a much lower capacity to transport tocotrienols, it has been previously reported that orally supplemented tocotrienol results in plasma tocotrienol concentration in the range 3 μM [16].

In our study, liver function was not altered due to supplementation of tamoxifen in combination with TRF. It seems that this combination is well tolerated.

The major strength of this study is that, it is the first experimental study that was conducted to investigate the effect of TRF, on survival of patients with early breast cancer. Furthermore, we had a complete follow-up of all of our patients at every point of assessment, and they also complied well to the treatment assigned to them.

The lack of randomization is a limitation in this study. However the two groups were balanced with respect to demographic and tumor characteristics. In addition a notable weakness in this study is that we had a relatively small sample size with low number of outcomes, possibly resulting from using breast cancer specific death as our primary outcome leading to a low power to detect statistically significant differences in the outcome between the two groups.

## Conclusions

Results from this study are not sufficient to indicate a significant association between adjuvant tocotrienol therapy and breast cancer survival in women with early breast cancer. It is important

to note that there is accruing evidence suggesting that tocotrienols have anti-cancer effects. Moreover, tamoxifen and tocotrienol *in vitro* have demonstrated synergy. Although there was a 60% lower mortality in the intervention group, this result was not statistically significant. Hence, a large randomized trial is certainly warranted in the near future to establish if tocotrienol adjuvant therapy can significantly improve recurrence and/or mortality.

## Abbreviations

TNM = tumor node metastases; α-TTP = alpha-tocopherol transfer protein; CBC = complete blood count; HPLC = high performance liquid chromatography; LDL = low density lipoprotein; RBC = red blood cell; TRF = tocotrienol rich fraction; TAP = tocopherol-associated proteins.; ER = estrogen receptor.

## Competing interests

The authors declare that they have no competing interest. Hovid Sdn. Bhd. absolutely did not have any influence in the trial designing, patient recruitment, data collection, analysis and reporting.

## Authors' contributions

The authors' responsibilities were as follows - KN, KRS, PAG and GAR: study design, data collection, statistical analyses, interpretation of data and KN, KRS, PAG, GAR and SDV in manuscript writing.

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**Figure legends**

**Figure 1.** Flow diagram of the progress through the phases parallel trial of two groups (enrolment, intervention allocation, follow-up and data analysis).

**Figure 2.** Breast cancer specific survival in intervention versus control group.

**Figure 3.** The α-tocopherol, α-, γ- and δ-tocotrienol concentrations increased significantly after 5years for TRF supplemented group. * ($p<0.05$).

**Table 1: Baseline demographic and tumor profile of 240 breast cancer patients with estrogen receptor positive tumor**

| | Tocotrienol + Tamoxifen (n=120) | Tamoxifen only (n=120) | P value |
|---|---|---|---|
| **Age (years)** | 48.5 (5.2) | 49.1 (5.9) | 0.30 |
| **Ethnicity**: | | | |
| Malay | 50.0 (60) | 46.7 (56) | 0.61 |
| Chinese | 30.0 (36) | 37.5 (45) | 0.22 |
| Indian | 20.0 (24) | 15.8 (19) | 0.40 |
| **Stage of breast cancer**: | | | 0.07 |
| 1 | 60.8 (73) | 49.2 (59) | |
| 2 | 39.2 (47) | 50.8 (61) | |
| **Involvement of lymph node**: | | | |
| Yes | 58.3 (70) | 62.5 (75) | 0.51 |
| Number of lymph nodes involved* | 1.5 (3.1) | 1.1 (1.9) | 0.15 |

Continuous variables are expressed as means (standard deviations) and categorical variables as percentages (numbers).

* Only includes patients with lymph node involvement.

**Table 2: Tocotrienol intake and risk of breast cancer specific death/ recurrence in women with early breast cancer and estrogen receptor positive tumors receiving tamoxifen**

| | Type of treatment received along with tamoxifen | |
|---|---|---|
| | Placebo (n=120) | Tocotrienol (n=120) |
| **Breast Cancer Related Death** | | |
| No of patients [n (%)] | 6 (5.0) | 2 (1.7) |
| Crude Hazard Ratio | 1.00 | 0.33 (95% CI: 0.07 to 1.61) |
| Adjusted Hazard Ratio* | 1.00 | 0.40 (95%CI: 0.08 to 2.05) |
| **Local/ Systemic Recurrence of Breast Cancer** | | |
| No of patients [n (%)] | 20 (16.7) | 16 (13.3) |
| Crude Hazard Ratio | 1.00 | 0.80 (95% CI: 0.41 to 1.54) |
| Adjusted Hazard Ratio* | 1.00 | 0.84 (95%CI: 0.43 to 1.65) |

***** Adjusted for age (continuous), ethnicity (Malay, Chinese, Indian), TNM stage (stage 1, stage 2), and lymph node involvement (yes, no).

## Table 3 Blood parameters

| Parameters | Range | Day 0 | Year 1 | Year 2 | Year 3 | Year 4 | Year 5 |
|---|---|---|---|---|---|---|---|
| *Group* | | (Mean ± SD) | | | | | |
| **White Blood Count** | | | | | | | |
| *Placebo + Tamoxifen* | 4-11 | 6.29 ± 2.09 | 6.77 ± 1.79 | 7.06 ± 2.07 | 7.11 ± 2.37 | 7.22 ± 7.22 | 7.36 ± 7.36 |
| *TRF + Tamoxifen* | ($X10^9$/L) | 5.86 ± 2.01 | 5.81 ± 1.59 | 5.86 ± 1.78 | 6.28 ± 1.92 | 6.33 ± 1.76 | 6.46 ± 1.62 |
| **Red Blood Count** | | | | | | | |
| *Placebo + Tamoxifen* | 3.8 -5.8 | 4.48 ± 0.48 | 4.46 ± 0.44 | 4.41 ± 0.40 | 4.49 ± 0.46 | 4.43 ± 0.54 | 4.55 ± 0.53 |
| *TRF + Tamoxifen* | ($X10^{12}$/L) | 3.95 ± 0.81 | 4.24 ± 0.41 | 4.27 ± 0.34 | 4.28 ± 0.34 | 4.27 ± 0.45 | 4.28 ± 0.40 |
| **Haemoglobin** | | | | | | | |
| *Placebo + Tamoxifen* | 11.5-16.5 | 12.49 ± 1.28 | 12.40 ± 1.18 | 12.63 ± 1.16 | 12.66 ± 0.96 | 12.61 ± 0.92 | 12.70 ± 0.96 |
| *TRF + Tamoxifen* | (g/dL) | 11.79 ± 2.48 | 12.71 ± 1.13 | 12.67 ± 0.93 | 12.72 ± 0.99 | 12.65 ± 1.27 | 12.77 ± 1.01 |
| **Haematocrit** | | | | | | | |
| *Placebo + Tamoxifen* | 37 - 47 | 38.90 ± 3.27 | 38.34 ± 2.56 | 38.53 ± 2.82 | 38.48 ± 2.68 | 37.44 ± 8.69 | 38.69 ± 3.06 |
| *TRF + Tamoxifen* | (%) | 35.51 ± 7.30 | 38.76 ± 4.31 | 37.96 ± 4.32 | 38.68 ± 2.97 | 38.36 ± 4.19 | 38.63 ± 3.27 |
| **Lymphocyte** | | | | | | | |
| *Placebo + Tamoxifen.* | 20-45 | 33.03 ± 6.62 | 33.37 ± 6.21 | 34.30 ± 8.13 | 35.19 ± 6.27 | 34.82 ± 8.32 | 35.48 ± 7.20 |
| *TRF + Tamoxifen* | (%) | 31.42 ± 8.35 | 33.79 ± 9.41 | 33.22 ± 8.58 | 34.65 ± 8.68 | 34.59 ± 6.63 | 36.39 ± 9.58 |
| **Platelet Count** | | | | | | | |
| *Placebo + Tamoxifen.* | 150 - 400 | 221.52 ± 60.59 | 221.48 ± 46.09 | 222.19 ± 61.39 | 238.10 ± 61.63 | 250.00 ± 61.87 | 260.10 ± 57.85 |
| *TRF + Tamoxifen* | ($X10^9$/L) | 221.16 ± 89.81 | 217.89 ± 50.74 | 217.63 ± 58.52 | 226.13 ± 49.69 | 247.74 ± 76.05 | 243.07 ± 61.02 |

The mean values of the results from the biochemical screening tests on day 0, year 1, 2, 3, 4 and 5 of tocotrienol rich fraction (TRF) plus tamoxifen supplemented group and placebo plus tamoxifen supplemented group.

## Table 4. Liver Function Tests

| Parameters | Range | **Day 0** | **Year 1** | **Year 2** | **Year 3** | **Year 4** | **Year 5** |
|---|---|---|---|---|---|---|---|
| *Group* | | (Mean ± SD) | | | | | |
| **Total Protein** | | | | | | | |
| *Placebo + Tamoxifen* | 66 - 87 | 76.68 ± 5.04 | 76.72 ± 5.89 | 77.56 ± 5.12 | 77.32 ± 4.55 | 75.48 ± 5.28 | 77.08 ± 5.10 |
| *TRF + Tamoxifen* | g/L | 76.03 ± 4.71 | 76.31 ± 5.33 | 76.41± 5.07 | 76.82 ± 4.55 | 77.08 ± 4.65 | 76.54 ± 4.82 |
| **Albumin** | | | | | | | |
| *Placebo + Tamoxifen.* | 35 - 50 | 41.48 ± 3.78 | 41.72 ± 3.80 | 40.84 ± 7.14 | 42.60 ± 4.29 | 41.48 ± 3.81 | 41.40 ± 7.46 |
| *TRF + Tamoxifen* | g/L | 41.28 ± 3.24 | 41.36 ± 3.69 | 41.05 ± 3.64 | 42.00 ± 4.13 | 41.69 ± 4.46 | 41.92 ± 4.07 |
| **Total Bilirubin** | | | | | | | |
| *Placebo + Tamoxifen* | < 21 | 7.80 ± 3.81 | 7.80 ± 4.17 | 8.40 ± 3.58 | 9.16 ± 3.98 | 8.12 ± 3.70 | 8.72 ± 4.09 |
| *TRF + Tamoxifen* | umol/L | 8.00 ± 3.94 | 7.74 ± 3.70 | 7.74 ± 3.88 | 8.15 ± 4.63 | 7.95 ± 3.72 | 8.16 ± 3.89 |
| **Alkaline Phosphatase** | | | | | | | |
| *Placebo + Tamoxifen* | 42 - 98 | 65.08 ± 17.09 | 63.88 ± 15.21 | 64.56 ± 19.58 | 66.80 ± 17.14 | 62.12 ± 15.14 | 65.28 ± 18.92 |
| *TRF + Tamoxifen* | U/L | 66.67 ± 18.55 | 62.51 ± 20.15 | 66.18 ± 20.41 | 64.74 ± 18.44 | 67.79 ± 20.06 | 68.42 ± 19.92 |
| **Alanine Transminase** | | | | | | | |
| *Placebo + Tamoxifen* | < 32 | 28.28 ± 22.69 | 34.04 ± 50.06 | 23.28 ± 13.87 | 27.72 ± 25.74 | 32.28 ± 48.75 | 24.28 ± 18.16 |
| *TRF + Tamoxifen* | U/L | 32.18 ± 29.63 | 32.85 ± 18.93 | 31.69 ± 20.55 | 32.13 ± 28.04 | 29.77 ± 15.25 | 28.79 ± 15.14 |

The mean values of the results from the Liver function tests on day 0, year 1, 2, 3, 4 and 5 of tocotrienol rich fraction (TRF) plus tamoxifen supplemented group and placebo plus tamoxifen supplemented group.

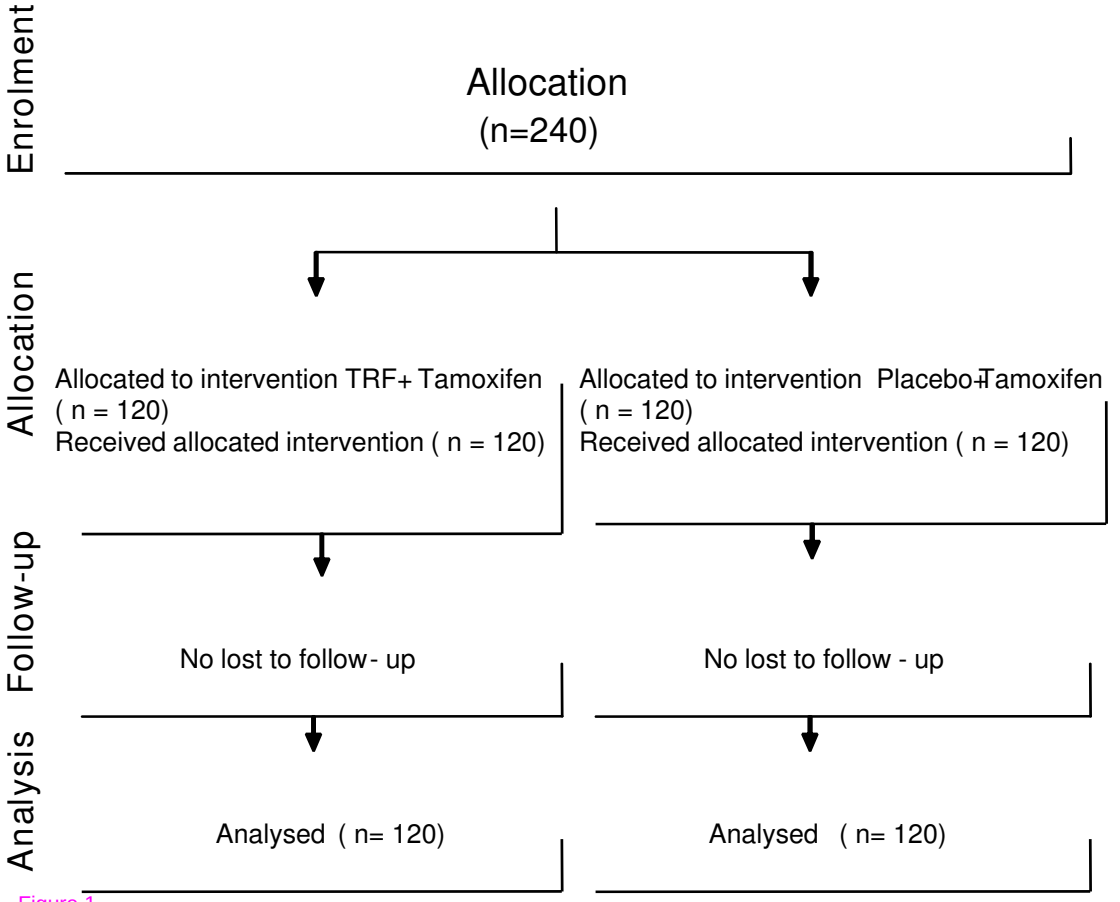

Enrolment
Allocation
(n=240)
Allocation
Allocated to intervention TRF+ Tamoxifen
( n = 120)
Received allocated intervention ( n = 120)
Allocated to intervention Placebo+Tamoxifen
( n = 120)
Received allocated intervention ( n = 120)
Follow-up
No lost to follow- up
No lost to follow - up
Analysis
Analysed ( n= 120)
Analysed ( n= 120)

Figure 1

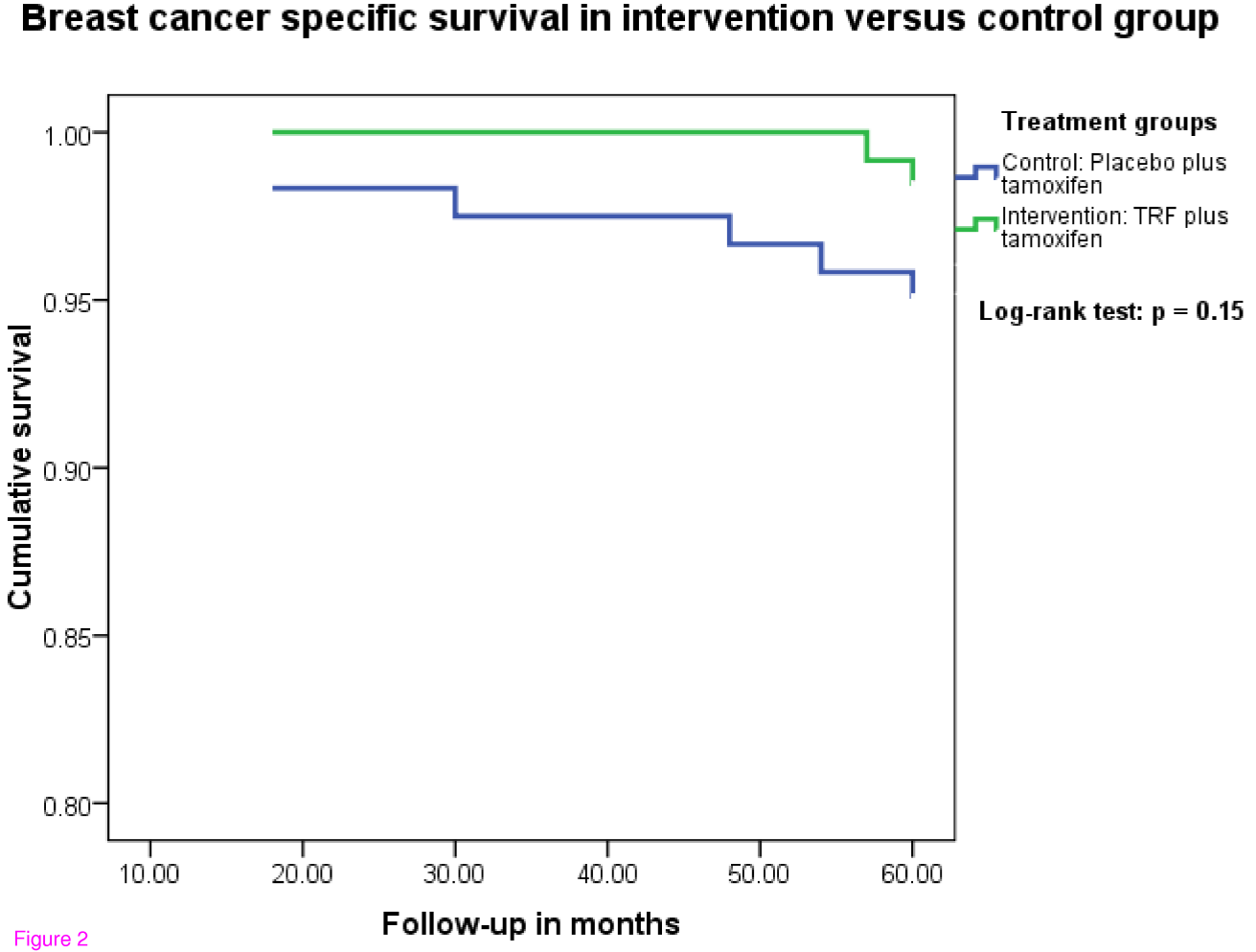
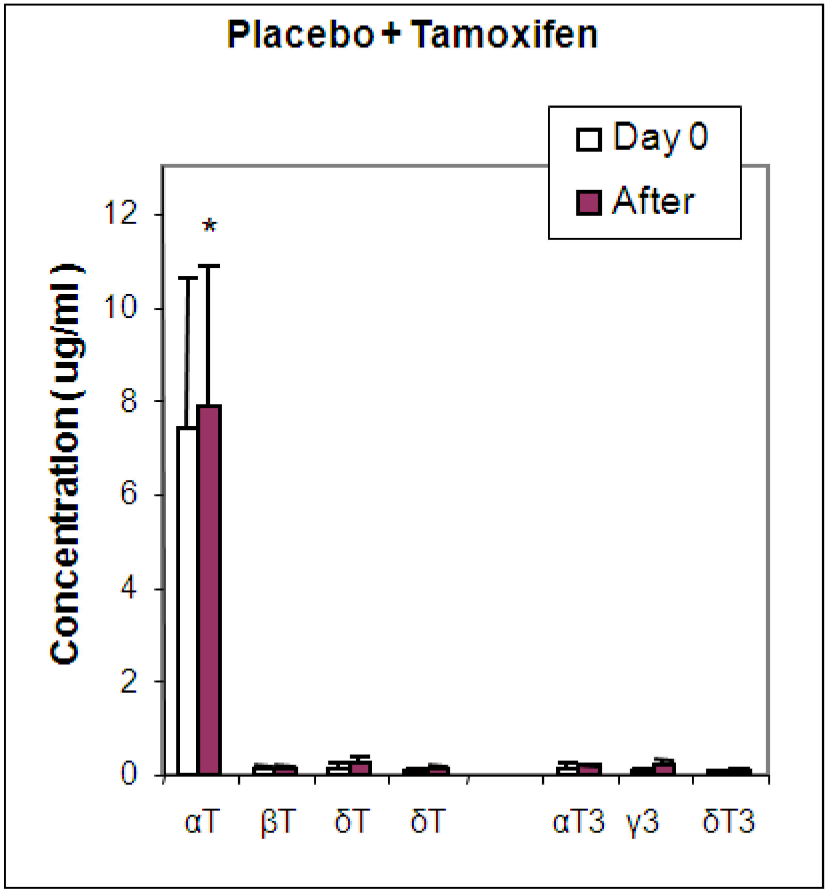
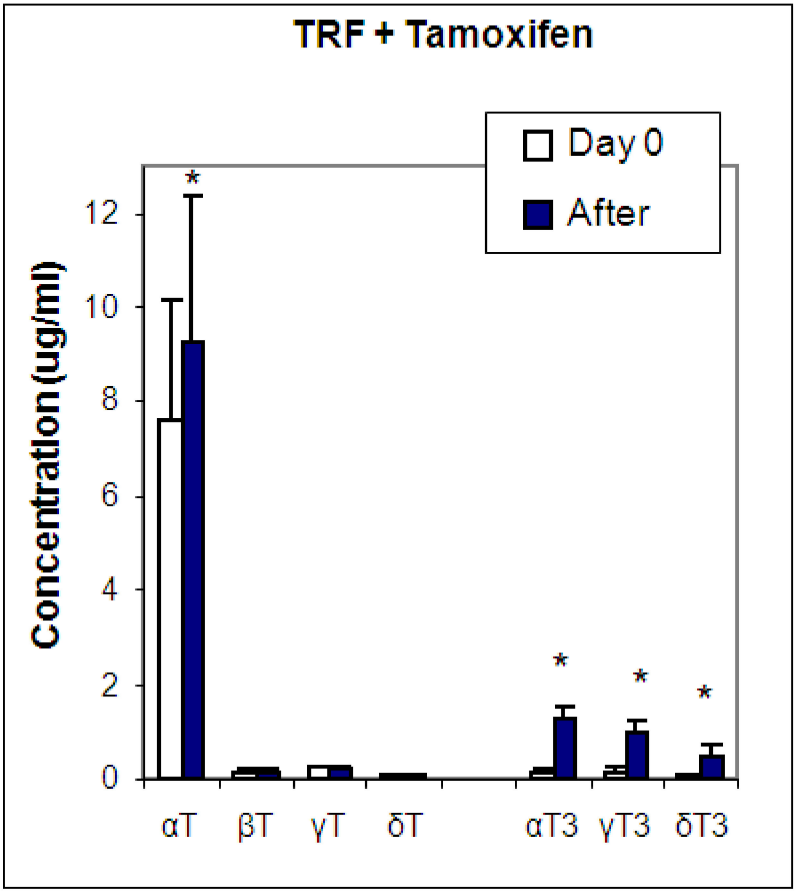

Breast cancer specific survival in intervention versus control group
Cumulative survival
1.00
0.95
0.90
0.85
0.80
10.00
20.00
30.00
40.00
50.00
60.00
Follow-up in months
Treatment groups
Control: Placebo plus tamoxifen
Intervention: TRF plus tamoxifen
Log-rank test: p = 0.15

Figure 2




Note: After – Completion of 5 years

Figure 3