**Adjuvant Aromatase Inhibitors or Tamoxifen Following Chemotherapy for Perimenopausal Breast Cancer Patients**

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**Abstract**

**Background.** Benefit of adjuvant aromatase inhibitors (AI) versus tamoxifen has been investigated in randomized clinical trials for premenopausal and postmenopausal patients with early, estrogen receptor positive (ER+) breast cancer. The optimal endocrine treatment for chemotherapy-treated perimenopausal women, who generally develop chemotherapy-induced amenorrhea, is uncertain.

**Methods.** All Dutch women who received adjuvant chemotherapy and endocrine treatment for stage I-III, ER+ (>10% positive cells), invasive breast cancer, diagnosed between 2004-2007, were identified through the Netherlands Cancer Registry. Included women were considered perimenopausal based on an age at diagnosis of 45-50 years (n=2,295). For each patient AI treatment duration relative to total endocrine treatment duration was calculated. Predominantly tamoxifen-treated patients (AI<25%) were compared with those receiving AI and tamoxifen for a similar duration (AI 25%-75%) and those mostly using AI (AI>75%). Adjusted hazard ratios (HRs) for recurrence-free survival (RFS) and overall survival (OS) were calculated using time-dependent Cox regression.

**Results.** After an average follow-up of 7.6 years, 377 RFS events occurred. Women mostly receiving AI (AI>75%) had the best RFS (adjusted-HR = 0.63; 95% confidence interval = 0.46-0.86) followed by those receiving AI 25%-75% (adjusted-HR = 0.85; 95% confidence interval = 0.65-1.12), when compared to predominantly tamoxifen-treated women. Trend analyses showed that every 10% increase in AI-endocrine treatment ratio reduced RFS event risk with 5% (2-sided Ptrend = 0.002). In total, 236 deaths occurred, hazard ratios for OS showed similar trends.

**Conclusion.** These results suggest that the best adjuvant endocrine treatment for chemotherapy-treated, ER+ breast cancer patients diagnosed aged 45-50 years, consists of mainly AI followed by a switch strategy and mainly tamoxifen.

**Keywords:** breast cancer, ER, endocrine therapy, tamoxifen, aromatase inhibitors, chemotherapy-induced amenorrhea, chemotherapy-induced menopause, recurrence free survival, overall survival, time dependent analysis

**LIST OF ABBREVIATIONS**

**AI**  Aromatase inhibitor

**AC** Doxorubicin + cyclofosfamide

**BMI** Body Mass Index

**CI** Confidence interval

**DA** Doxorubicin + docetaxel

**DFS** Disease-free survival

**ER** Estrogen receptor

**FAC/CAF** 5-FU + doxorubicin + cyclofosfamide

**FEC/CEF** 5-FU + epirubicin + cyclofosfamide

**GnRH** Gonadotropin-Releasing Hormone agonist

**HER2** Human Epidermal growth factor Receptor 2

**HR** Hazard ratio

**n** Number

**OA** Ovarian ablation

**OS** Overall survival

**PR** Progesterone receptor

**pT-stage** Pathologic T-stage

**RFS** Recurrence-free survival

**TAC**  Docetaxel + doxorubicin + cyclofosfamide

Adjuvant endocrine treatment for premenopausal women with estrogen and/or progesterone receptor positive breast cancer has long consisted of tamoxifen. The SOFT/TEXT trial results have challenged this standard by showing that tamoxifen plus ovarian ablation (OA) improve disease-free survival and overall survival at 8 years when compared to tamoxifen alone, while exemestane, an aromatase inhibitor (AI), plus OA led to even higher rates of freedom of recurrence. [1-3](#_ENREF_1)

For postmenopausal patients, five years of tamoxifen followed by an AI for two to three years, tamoxifen for two to three years followed by an AI for up-to five-years or upfront AI for five-years are common endocrine treatment regimens.[4](#_ENREF_4),[5](#_ENREF_5)

For endocrine treatment purposes women are usually considered premenopausal until definite menopause occurs. Menopausal status at diagnosis is often used as the basis for endocrine treatment allocation. However, the menopausal transition or perimenopause, is a gradual process caused by the continuing decline in ovarian reserve.[6](#_ENREF_6) Perimenopausal women experience, amongst others, irregular cycle lengths and an increased susceptibility for the ovarian suppressive effects of chemotherapy. [7](#_ENREF_7)

Chemotherapy-induced amenorrhea is a well-known side-effect that on average occurs in 77% (95% confidence interval (CI) 71-83%) of premenopausal women treated with chemotherapy ≥40 years of age.[8](#_ENREF_8) While considered an adverse event of chemotherapy, chemotherapy-induced amenorrhea is associated with improved outcome in women with hormone receptor positive breast cancer diagnosed <40 years of age.[9](#_ENREF_9) Amenorrhea in women ≥40 years post-chemotherapy often proves to be irreversible and is also called chemotherapy-induced menopause. However, estrogen depletion increases the production of gonadotropins, which stimulate the ovaries and can cause ovarian function recovery.[10](#_ENREF_10)

Ovarian function recovery can occur during tamoxifen as well as AI treatment and may initially remain unnoticed.[11](#_ENREF_11) While tamoxifen remains an active drug in the presence of ovarian function recovery, its occurrence renders AI treatment ineffective.[12](#_ENREF_12) It is therefore unclear if AI treatment in perimenopausal women with chemotherapy-induced amenorrhea is safe or whether tamoxifen should be preferred instead. The current study aims to determine the optimal endocrine treatment (AI or tamoxifen) for chemotherapy treated women who were 45-50 years at breast cancer diagnosis, likely perimenopausal and thus at a high risk of developing chemotherapy–induced amenorrhea or menopause.

**Methods**

**Patient selection**

Through the Netherlands Cancer Registry we identified all Dutch women who were 45-50 years old when diagnosed with a T1-4NanyM0 estrogen receptor positive (ER+) primary breast cancer between 2004-2007. Eligible women had no history of prior malignancy and received adjuvant chemotherapy and endocrine treatment.

From its establishment in 1989, the prospective population-based Netherlands Cancer Registry has registered all newly diagnosed, histologically confirmed cancers. For this study additional information on Body Mass Index (BMI), treatments and disease recurrences were gathered. All data are obtained directly from patient hospital records by trained registrars. Vital status is acquired from the municipal population registry. Cause of death is not registered because of Dutch privacy regulations.

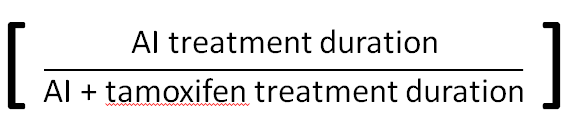
Registrars from the Netherlands Cancer Registry derived ER, progesterone receptor (PR) and HER2 status from local pathology reports. According to Dutch guidelines tumors were considered ER+ and/or PR+ when immunohistochemistry stained >10% of tumor cells positive.[13](#_ENREF_13) HER2 positivity was demonstrated by polymerase chain reaction, in situ hybridization or a 3+ score on immunohistochemistry.[14](#_ENREF_14)

**Ethics approval**

This project was approved by the Medical Ethical Committee of the Netherlands Cancer Institute – Antoni van Leeuwenhoek hospital (PTC12.1262/NBCP).

**Statistical analysis**

Because of frequent treatment switches between tamoxifen and AI, we used the AI-endocrine treatment ratio, as previously described, to determine the predominant endocrine treatment received.[15](#_ENREF_15) In short, at any event time during follow-up we calculated the AI-endocrine treatment ratio



x 100%.[15](#_ENREF_15)

For women with missing treatment start dates, stop date of previous treatment or date of diagnosis was used instead. For women with missing stop dates, the respective date of subsequent treatment start, disease recurrence, end of follow-up or death were used.

We used the AI-endocrine treatment ratio in a time-dependent manner to group patients into those mainly tamoxifen-treated (AI<25%), mostly AI-treated (AI>75%) orthose with roughly similar durations of tamoxifen and AI treatment (AI 25%-75%). In addition, we also evaluated trends by analyzing the AI-endocrine treatment ratio as a continuous variable.

Study endpoints were recurrence-free survival (RFS) and overall survival (OS). RFS was defined as time from cancer diagnosis to disease recurrence (ipsilateral, local, regional or distant) or death from any cause, whichever occurred first. OS was calculated as time from cancer diagnosis to death from any cause.[16](#_ENREF_16) However, time was left truncated at the start of first endocrine treatment. Patients without a RFS or OS event at the end of follow-up and those lost to follow-up were censored.

The association between the AI-endocrine treatment ratio and RFS and OS was assessed by Cox regression, adjusted for age at diagnosis, trastuzumab use, grade, number of positive lymph nodes, pT-stage, PR-status, HER2-status and OA. Due to low numbers, women for whom nodal status was not known (n=11) were left out of Cox regression analyses. Trastuzumab use and OA were used as time-dependent variables, using follow-up as the time scale. The proportional hazards assumption was tested using Schoenfeld residuals. If violated, an interaction between the covariate and follow-up time centered at 5-years was added to the model. Treatment group-specific survival functions were estimated by multivariable Cox models and plotted at the average covariate values. Ninety five percent CIs for 5-year RFS and OS rates were estimated with 1000 bootstrap samples.

Sensitivity analyses were performed by adjusting for BMI, number of treatment switches, total endocrine treatment duration and type of first endocrine treatment received (tamoxifen vs AI). In addition, calculations were done excluding women with missing start and stop dates of the first endocrine treatment and excluding women who did not receive ovarian ablation. Furthermore, analyses were repeated using alternative categorizations of the AI-treatment ratio. Lastly, we investigated whether AI treatment benefit differed by PR and HER2-status.

# Statistical analyses were performed using R version 3.6.3 and Stata SE 15.

**Results**

**Study population**

We identified 2,295 women who were 45-50 years of age, when diagnosed between 2004-2007, with an ER+ invasive breast cancer. We included 204 (8.9%) of these women in a previous study on the optimal endocrine treatment of ER+/HER2+ breast cancer patients.[15](#_ENREF_15)

All patients received adjuvant chemotherapy and endocrine treatment. Endocrine treatment consisted of tamoxifen and/or AI. Most women started on tamoxifen (1,903 of 2,295; 82.9%). The average duration of endocrine treatment was 5.5 years. For the 1,504 of 2,295 (65.5%) women who received endocrine treatment beyond five years, average treatment duration was 6.5 years. Only 2 of these women received therapy for 10 or more years.

**Supplementary Figure 1** summarizes details on missing treatment start and stop dates.

The majority of patients switched between endocrine treatment modalities (**Supplementary Figure 2A-C**). Only 34.7% of patients (796 of 2,295) received one type of treatment. Of these non-switchers, 56.3% (448 of 796) received tamoxifen and 43.7% received an AI (348 of 796) (**Supplementary Table 1**). Baseline characteristics are shown per treatment group as defined by the AI-endocrine treatment ratio at the end of follow-up (AI<25%, 25%≤AI≤75% and AI>75%) (**Table 1)**. At that time, 47.5% (1,091 of 2,295) of patients had received an AI for 25%-75% of their endocrine treatment duration, 27.2% (624 of 2,295) had received an AI<25% and 25.3% (580 of 2,295) of patients had received an AI>75%. Most women had pT2 (1,178 of 2,239; 52.6%) and grade II-III (1,801 of 2,085; 86.4%) tumors. Metastases to ≥1 lymph node were present in 72.1% (1,647 of 2,284) of women. Of all the ER+ tumors, 87.3% (1,908 of 2,185) co-expressed PR. HER2-positivity was observed in 6.9% (39 of 567) and 3.9% (39 of 990) of women treated with an AI<25% and 25%≤AI≤75% of their endocrine treatment duration versus 38.2% (194 of 508) in women who received an AI>75% of the time, respectively. Chemotherapy regimens contained an anthracycline in 96.6% (2,218 of 2,295) of all women (**Table 1**).

**Recurrence-free survival**

During an average follow-up time of 7.6 years, a total of 377 RFS events were observed, most of these (71.1%, 268 of 377) involved distant metastases (**Supplementary Table 2**). At the end of follow-up, 29.6% (185 of 624) of the women who received an AI<25% of their endocrine treatment duration, experienced a disease recurrence compared to 10.8% (118 of 1,091) and 12.8% (74 of 580) in women treated 25%≤AI≤75% and AI>75%, respectively.

When compared to women who were treated with an AI<25% of their endocrine treatment duration, women who received an AI>75% had a statistically significant improvement in RFS with an adjusted-HR of 0.63 (95% CI = 0.46-0.86) and an adjusted five-year RFS rate of 94.5% (95% CI = 93.0%-96.8%) versus 91.4% (95% CI = 90.2%-94.7%) (**Figure 1, Table 2**). Women treated 25%≤AI≤75% did not have a different risk of RFS compared to women treated with AI<25% (adjusted-HR = 0.85; 95% CI = 0.65-1.12; adjusted five-year RFS rate of 92.3% (95% CI = 90.6%-95.3%) versus 91.4% (95% CI = 90.2%-94.7%). When the AI-endocrine treatment ratio was used as a continuous variable, an adjusted-HR of 0.95 (95% CI = 0.91-0.98; Ptrend = 0.002) was observed indicating that the risk of an RFS event is reduced by 5% for each additional 10% increase in AI-endocrine treatment ratio.

**Overall survival**

During an average follow-up time of 7.7 years 236 deaths were observed. Average follow-up times differed slightly by AI-endocrine treatment ratio and were 7.1 years, 7.8 years and 8.0 years for women who were treated with an AI<25%, AI 25%-75% and AI>75%, respectively.

Compared to women with an AI-endocrine treatment ratio <25%, women who received an AI>75% experienced better OS (adjusted-HR = 0.50, 95% CI = 0.34-0.74; adjusted five-year OS rate 97.3% (95% CI = 96.4%-98.4%) versus 94.6% (95% CI = 93.6%-96.1%), respectively) (**Figure 2, Table 3**). Overall survival was also better during the first few years of follow-up for women who were treated with 25%≤AI≤75% compared to women who received AI<25% (adjusted five-year OS rate of 97.6% (95% CI = 97.0%-98.4%) versus 94.6% (95% CI = 93.6%-96.1%), respectively) (**Figure 2**). The HRs between women who were treated with an 25%≤AI≤75% versus AI<25% vary with time because the proportional hazards assumption was violated. At 5-years, the risk of dying was reduced by approximately 70% when receiving 25%≤AI≤75% compared to AI<25% (adjusted-HR = 0.32, 95% CI = 0.21-0.49 at 5 years). After year 5, the relative risk of dying increased for each additional one-year increment in follow-up time. This can be calculated as follows:

HR=exp{(ln(0.32)+(follow-up time – 5 years)\*ln(1.42)}

For example, at 6 years of follow-up women who received an 25%≤AI≤75% versus AI<25% had an adjusted-HR of 0.45 (95% CI = 0.33-0.66) (**Figure 2, Table 3**).

When the AI-endocrine treatment ratio was used as a continuous variable, an adjusted-HR of 0.90 (95% CI = 0.86-0.93; Ptrend <0.001) was observed, indicating that the risk of dying is reduced by 10% for each additional 10% increase in AI-endocrine treatment ratio.

**Sensitivity analyses**

The effects of AI-treatment on RFS and OS, adjusted for BMI, total endocrine treatment duration, number of treatment switches, type of first endocrine treatment received and including only women whose start or stop date of first endocrine treatment was known, were comparable to the AI treatment effect from the main models (**Supplementary Table 3**). Separate analyses for the 498 women who ever received OA at any stage during endocrine treatment were again very similar to the overall AI treatment effect (**Supplementary Table 4**). Analyses using alternative AI-endocrine treatment ratio cut-offs (AI 0%-30%-70%-100%, AI 0%-40%-60%-100%, AI 0%-50%-100%, AI 0%-100%), also showed similar patterns (**Supplementary Table 5**).

Lastly, results for OS and RFS did not differ by PR (**Supplementary Table 6**) and HER2 status (**Supplementary Table 7**) (all Pinteraction >0.05).

**Discussion**

Our study represents a rigorous, well-annotated prospective, population-based cohort study in a well-defined patient population. The data are derived from the Netherlands Cancer Registry, which is known to provide highly accurate and complete cancer incidence.

Our study is the first to systematically address the relative efficacy of adjuvant aromatase inhibitors (AI) over tamoxifen in breast cancer patients 45-50 years at diagnosis who are likely perimenopausal and commonly excluded from randomized-controlled trials, including the SOFT and TEXT trials where only 31.9% of all patients were 45-49 years of age.[3](#_ENREF_3) Therefore, all current recommendations on adjuvant endocrine therapy for perimenopausal women are based on extrapolations of data from premenopausal or postmenopausal women. Our results emphasize the clinically relevant beneficial effect of AI treatment after chemotherapy in this particular age group and support the abovementioned extrapolation reassuring both breast cancer oncologists and patients worldwide.

The aim of the current study was to assess whether chemotherapy-treated women, 45-50 years at breast cancer diagnosis, who are likely perimenopausal and thus at a high risk of developing amenorrhea or menopause, derive more benefit from AI treatment compared to tamoxifen. We found that RFS and OS improve considerably with an increasing AI-endocrine treatment ratio, that is, the longer a woman is treated with an AI compared with tamoxifen.

The findings of our study are in line with posthoc analyses conducted within the monotherapy arms of the Breast International Group (BIG) 1-98 trial.[17](#_ENREF_17) In this study the authors investigated the effect of tamoxifen and letrozole on disease-free survival (DFS) in women with either chemotherapy-induced menopause or recent natural menopause (aged ≤55 years). After correction for prognostic factors, a statistically significant DFS benefit of AIs versus tamoxifen was observed in the women with chemotherapy-induced menopause (adjusted-HR = 0.21 95% CI = 0.05-0.94).[17](#_ENREF_17)

A recent meta-analysis on the incidence and risk factors of chemotherapy induced-amenorrhea found that 77% (95% CI = 71%-83%) of premenopausal, chemotherapy treated women ≥40 years of age experience amenorrhea, irrespective of the type of chemotherapy administered.[8](#_ENREF_8) Overall, risk factors for chemotherapy-induced amenorrhea were age at diagnosis >40 years and tamoxifen treatment following chemotherapy.[8](#_ENREF_8),[18](#_ENREF_18)

Although chemotherapy-induced amenorrhea is considered a chemotherapeutic side effect, its occurrence has an independent positive effect on the outcome of women diagnosed with hormone sensitive breast cancer under the age of 40 years.[9](#_ENREF_9),[19](#_ENREF_19),[20](#_ENREF_20)

The cessation of menses in women with chemotherapy-induced amenorrhea ≥40 years of age often proves permanent.[21](#_ENREF_21) In some cases, however, amenorrhea is temporary and ovarian function recovery, either clinical (menstruation or pregnancies) or subclinical (FSH/LH/E2 blood levels in the premenopausal range), occurs. Ovarian function recovery after chemotherapy-induced amenorrhea typically takes place within the first two years following chemotherapy.[22](#_ENREF_22) Yet cases of ovarian function recovery have been described many years after finishing chemotherapeutic treatment.[10](#_ENREF_10)

Consistent with the BIG 1-98 trial we found that AI treatment improves the outcome of women who are at increased risk for chemotherapy-induced amenorrhea, even though AIs are partly responsible for the ovarian function recovery that diminishes their anticancer effect.[17](#_ENREF_17) Indeed, one study found a 2-year disease free survival of 82% in women with ovarian function recovery compared to 100% for women without recovery of ovarian function after AI treatment (HR = 9.3, 95% CI = 3.3-48; P=0.04).[23](#_ENREF_23) Other studies, however, were unable to observe a difference in outcome.[9](#_ENREF_9)Unfortunately at this time, it is not possible to predict which women will experience ovarian function recovery after chemotherapy-induced amenorrhea.[22](#_ENREF_22),[24](#_ENREF_24),[25](#_ENREF_25)

The weight of the evidence suggests that a younger age at chemotherapy initiation predicts for future occurrence of ovarian function recovery. Biochemical ovarian functionmonitoring is therefore highly recommended in young patients on AI treatment. Caution is needed when selecting the appropriate methodology because some assays may not be sensitive enough or interact with the metabolites of steroidal AIs .[26](#_ENREF_26)

Our study has some limitations. First, we lack information about the women’s actual menopausal status, both preceding and following chemotherapy. However, based on the age restriction of our study population (45-50 years) it is very likely that a large part of the women in our cohort were perimenopausal prior to chemotherapy initiation.[27](#_ENREF_27) Hence, our assumption that these women are at an increased risk for chemotherapy-induced amenorrhea is probably justified since age at diagnosis is a reliable predictive factor.[8](#_ENREF_8) In addition, because of their age at diagnosis, they are highly likely to experience permanent amenorrhea.[21](#_ENREF_21),[22](#_ENREF_22),[24](#_ENREF_24),[25](#_ENREF_25)

Our assumption of increased risk for chemotherapy-induced amenorrhea is further supported by the high percentage of women in our cohort who received anthracycline or cyclophosphamide-containing chemotherapy regimens. Since besides age at diagnosis, the abovementioned chemotherapy regimens are known to increase the risk of developing chemotherapy-induced amenorrhea.[8](#_ENREF_8)

Furthermore, some of the women in our cohort may have been postmenopausal prior to chemotherapy initiation. Since it is known from literature that post-menopausal women derive a clinically significant benefit from AI treatment, inclusion of these women in our cohort may have enhanced the AI treatment effect. However, we believe the putative enhancement to be very small, since the number of postmenopausal women at diagnosis is expected to be less than 10%.[27](#_ENREF_27) In addition, women could have become postmenopausal due to bilateral oophorectomy performed for benign causes. However, the possible effect on our results is expected to be negligible since, based on information derived from Statistics Netherlands and the nationwide network and registry of histo- and cytopathology in The Netherlands, <1% of women aged 45-50 years had undergone bilateral oophorectomy between 2004-2007 in the Netherlands.[28](#_ENREF_28),[29](#_ENREF_29)

Unfortunately, we also lack information on side effects and patient adherence. Side effects are very common among women who receive endocrine treatment and may cause treatment discontinuation.[3](#_ENREF_3) Women with hormone receptor positive breast cancer should be encouraged to adhere to their assigned endocrine treatment, due to its observed effectiveness. In SOFT/TEXT the proportion of women who discontinued endocrine treatment was somewhat larger in the exemestane plus ovarian function suppression group when compared to the tamoxifen plus ovarian function suppression group (23.7% versus 19.3%).[3](#_ENREF_3) Here we propose that an age at diagnosis of 45-50 years, in combination with having received adjuvant chemotherapy, are clinical variables that may be a great motivation for patients to adhere to an AI because of its superior efficacy when compared to tamoxifen. Development and application of additional biomarkers and/or clinical profiles that can accurately predict side effects and therapy resistance would be a tremendous aid to oncologists in selecting the most appropriate treatment for each breast cancer patient.

Furthermore, we found that 194 of 508 (38.2%) women in our predominantly AI treated (AI>75%) patient subset were HER2+, compared to 6.9% and 3.9% in the predominantly tamoxifen treated (AI<25%) and AI-intermediate groups (25%≤AI≤75%). Women whose tumors co-express ER and HER2 are considered high risk and have an unfavorable prognosis. However, the majority of women with ER+/HER2+ tumors in our cohort received both chemotherapy and trastuzumab (**Table 1**). Due to the effectiveness of trastuzumab in HER2+ breast cancers, this formerly poor prognostic group now has a favorable prognosis. Historically, AIs are favored over tamoxifen in high-risk perimenopausal and postmenopausal women with ER+ tumors. An enrichment of women with ER+/HER2+ breast cancers who received trastuzumab treatment may have enhanced the relative effectiveness of AI in our cohort. However, treatment effect did not differ by HER2 status (Pinteraction>0.05) with similar trends as observed in the main analysis, indicating that the effect of HER2 status on our results is at best small (**Supplementary Table 5**).

Our analyses may have suffered from confounding by indication, as women with amenorrhea post-chemotherapy are more likely to receive an AI when compared to women without amenorrhea, while development of chemotherapy-induced amenorrhea itself is a favorable prognostic factor. To address this issue, we analyzed the AI-endocrine treatment ratio in the 498 patients who had undergone OA, based on the assumption that predominantly patients without chemotherapy-induced amenorrhea received OA. Again, this showed the same trends as in the main analysis (**Supplementary Table 3**).

Although comparable to the updated SOFT/TEXT trial, follow-up times in our cohort are still relatively short, with an average follow-up of 7.6 years for RFS and 7.7 years for OS, respectively. Nevertheless, we observed better RFS and OS for women who mainly received an AI compared to those who mostly received tamoxifen, irrespective of HER2 status. Analysis after longer follow-up is part of our future plan in order to fully appreciate the effect of AIs on OS in the treatment of perimenopausal breast cancer patients.

It should also be noted that the Dutch population is predominantly white. Between 2004 and 2007, when the women in our cohort were diagnosed, only 7.7%-8.5% of all 45-50-year-old women living in the Netherlands were of non-Dutch, non-Western decent.[30](#_ENREF_30) Therefore, caution is advised, as our results may not directly translate to a predominantly non-White/non-Caucasian population.

Lastly, using the AI-endocrine treatment ratio metric was not meant to identify an optimal combination of AI and tamoxifen, but to optimally exploit the information provided by the large group of treatment switchers.

In conclusion, ER+ breast cancer patients diagnosed between 45 and 50 years of age derive statistically significant RFS and OS benefit from treatment with predominantly AIs after chemotherapy. AIs can therefore be considered for all women in this age group with chemotherapy-induced amenorrhea, under strict monitoring of ovarian function to detect early signs of ovarian function recovery. Patients should be instructed to contact their physician when clinical signs of ovarian function recovery occur, including amongst others vaginal bleeding and the cessation of menopausal symptoms. At this point biochemical assessment of menopausal status should be performed. In case of ovarian function recovery, ovarian suppression or ablation should be added to AI treatment. When deemed inappropriate, tamoxifen remains a suitable alternative for these women.

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**Notes**

**Role of the funder:** None of the funders had any influence on study design; data collection; and/or project management; data analysis, interpretation; or manuscript preparation, review or approval.

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**Data Availability**

The data that support the findings of this study are available from the Netherlands Cancer Registry, hosted by the Netherlands Comprehensive Cancer Centre (IKNL) but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of The Netherlands Comprehensive Cancer Centre (IKNL).

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**Table 1**. Baseline characteristics of all 2,295 ER+ breast cancer patients by AI-endocrine treatment ratio at the end of follow-up.a

|  |  |  |  |
| --- | --- | --- | --- |
| Characterisitic | AI<25% | 25%≤AI≤75% | AI>75% |
| Total, No. (%) | 624 (100) | 1,091 (100) | 580 (100) |
| Mean age (range), y | 47.4 (45-50) | 47.8 (45-50) | 47.9 (45-50) |
| pT-stage, No. (%)  1,1a,1b,1c  2  3  4,4a,4b,4c  Unknown | 232 (37.1)  333 (53.4)  39 (6.3)  8 (1.3)  12 (1.9) | 451 (41.3)  548 (50.3)  64 (5.9)  8 (0.7)  20 (1.8) | 226 (39.0)  297 (51.2)  29 (5.0)  4 (0.7)  24 (4.1) |
| Grade, No. (%)  I  II  III  Unknown | 69 (11.1)  282 (45.2)  211 (33.8)  62 (9.9) | 150 (13.8)  514 (47.1)  334 (30.6)  93 (8.5) | 65 (11.2)  243 (41.9)  217 (37.4)  55 (9.5) |
| Positive lymph nodes, No. (%)  0  1-3  4-9  >10  Unknown | 151 (24.2)  324 (51.9)  93 (14.9)  53 (8.5)  3 (0.5) | 312 (28.6)  566 (51.9)  153 (14.0)  56 (5.1)  4 (0.4) | 174 (30.0)  251 (43.2)  113 (19.5)  38 (6.6)  4 (0.7) |
| PR status, No. (%)  Negative  Positive  Unknown | 80 (12.8)  519 (83.2)  25 (4.0) | 97 (8.9)  938 (86.0)  56 (5.1) | 100 (17.2)  451 (77.8)  29 (5.0) |
| HER2 status, No. (%)  Negative  Positive  Unknown | 528 (84.6)  39 (6.3)  57 (9.1) | 951 (87.1)  39 (3.6)  101 (9.3) | 314 (54.1)  194 (33.5)  72 (12.4) |
| Trastuzumab, No. (%)  No  Yes | 603 (96.6)  21(3.4) | 1,068 (97.9)  23 (2.1) | 452 (77.9)  128 (22.1) |
| Ovarian ablation, No. (%)  Yesb  Surgery  GnRH  No | 155 (24.8)  54  121  469 (75.2) | 221 (20.3)  129  128  870 (79.7) | 122 (21.0)  69  80  458 (79.0) |
| Chemotherapyc, No. (%)  Yes  Anthracycline-based  Anthracycline- and taxane-based  Other  No | 624 (100)  481 (77.1)  118 (18.9)  25 (4.0)  0 (0) | 1,091 (100)  861 (78.9)  207 (19.0)  23 (2.1)  0 (0) | 580 (100)  367 (63.3)  184 (31.7)  29 (5.0)  0 (0) |

a The AI-endocrine treatment ratio is defined as the percentage of total endocrine treatment duration (AI+tamoxifen) that was spent on AI treatment. GnRH = Gonadotropin-Releasing Hormone agonist, AI = Aromatase inhibitor

b Numbers may not add-up because some patients received a GnRH prior to their surgery

cAnthracycline-based schedules: doxorubicin + cyclofosfamide (AC), 5-FU + epirubicin + cyclofosfamide (FEC/CEF), 5-FU + doxorubicin + cyclofosfamide (FAC/CAF). Anthracycline- and taxane-based schedules: docetaxel + doxorubicin + cyclofosfamide (TAC), doxorubicin + docetaxel (DA)

**Table 2.** Multivariable Cox regression for RFS in 2,284 ER+ breast cancer patients.

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | Events | Adjusted-HR (95% CI) | *Pa* |
| AI-endocrine treatment ratiob  AI<25%  25%≤AI≤75% AI  > 75% | 185  118  74 | 1.00 (Reference)  0.85 (0.65-1.12)  0.63 (0.46-0.86) | 0.27  0.004 |
| Age  Age\*(follow-up time - 5)c |  | 1.05 (0.98-1.11)  1.03 (1.00-1.05) | 0.11  0.01 |
| Trastuzumab  No  Yes | 375  2 | 1.00  0.56 (0.13-2.39) | 0.44 |
| Grade  I  II  III  Unknown | 60  26  138  153 | 0.45 (0.29-0.69)  0.61 (0.48-0.77)  1.00  1.38 (1.00-1.90) | <0.001  <0.001  0.05 |
| Positive lymph nodes  0  1-3  4-9  >10 | 70  156  89  62 | 1.00  1.37 (1.02-1.83)  2.29 (1.66-3.16)  4.55 (3.17-6.52) | 0.03  <0.001  <0.001 |
| pT-Stage  1, 1A, 1B, 1C  2  3  4, 4A, 4B, 4C  Unknown | 119  201  39  8  10 | 1.00  1.23 (0.98-1.55)  1.50 (1.02-2.19)  1.68 (0.79-3.57)  1.11 (0.56-2.18) | 0.07  0.04  0.18  0.75 |
| PR status  Negative  Positive  Unknown | 77  277  23 | 1.00  0.50 (0.38-0.64)  0.75 (0.46-1.20) | <0.001  0.23 |
| HER2 status  Negative  Positive  Unknown | 289  51  37 | 1.00  1.17 (0.83-1.66)  0.76 (0.53-1.09) | 0.36  0.14 |
| Ovarian ablation  No  Yes | 308  69 | 1.00  1.25 (0.95-1.64) | 0.10 |

a P values are based on a two-sided Wald test

b The AI-endocrine treatment ratio, included in the model as a time-dependent variable, is defined as the percentage of total endocrine treatment duration (AI+tamoxifen) spent on AI treatment. AI = Aromatase inhibitor, CI = Confidence interval, HER2 = Human Epidermal growth factor Receptor-2, HR = Hazard ratio, PR = Progesterone receptor, RFS = Recurrence free survival

c Interaction between age at diagnosis and follow-up time centered at 5-years was included to accommodate non-proportional hazards. At 5-years of follow-up two patients who differ one year in age have an adjusted-HR of 1.05, this means that the older patient has a 5% higher risk of a RFS-event compared to the younger patient. The HR increases by 3% for each additional year of follow-up, so for example, at 6-years of follow-up the adjusted hazard ratio equals exp{ln(1.05)+(follow-up time-5)\*ln(1.03)}= 1.08.

**Table 3.** Multivariable Cox regression for OS in 2,284 ER+ breast cancer patients.

|  |  |  |  |
| --- | --- | --- | --- |
| Characteristic | Events | Adjusted-HR (95% CI) | *Pa* |
| AI-endocrine treatment ratiob  AI<25%  25%≤AI≤75%  25%≤AI≤75%\*(follow-up time - 5)c  AI> 75% | 127  62  47 | 1.00  0.32 (0.21-0.49)  1.42 (1.12-1.80)  0.50 (0.34-0.74) | <0.001  0.003  <0.001 |
| Age |  | 1.05 (0.97-1.13) | 0.19 |
| Trastuzumab  No  Yes | 233  3 | 1.00  2.46 (0.72-8.40) | 0.15 |
| Grade  I  II  II\*(follow-up time - 5) c  III  Unknown | 43  12  82  99 | 0.33 (0.17-0.61)  0.55 (0.40-0.75)  1.22 (1.05-1.43)  1.00  1.41 (0.97-2.07) | <0.001  <0.001  0.009  0.07 |
| Positive lymph nodes  0  1-3  4-9  >10 | 43  93  56  44 | 1.00  1.34 (0.93-1.94)  2.31 (1.54-3.47)  4.76 (3.06-7.38) | 0.11  <0.001  <0.001 |
| pT-Stage  1, 1A, 1B, 1C  2  3  4, 4A, 4B, 4C  Unknown | 78  120  25  6  7 | 1.00  1.08 (0.81-1.45)  1.27 (0.79-2.04)  1.76 (0.73-4.20)  1.07 (0.48-2.41) | 0.57  0.31  0.20  0.85 |
| PR status  Negative  Positive  Unknown | 54  170  12 | 1.00  0.46 (0.34-0.64)  0.59 (0.31-1.12) | <0.001  0.11 |
| HER2 status  Negative  Positive  Unknown | 180  33  23 | 1.00  0.99 (0.63-1.54)  0.95 (0.61-1.49) | 0.98  0.85 |
| Ovarian ablation  No  Yes | 196  40 | 1.00  1.12 (0.79-1.59) | 0.50 |

a P values are based on a two-sided Wald test

b The AI-endocrine treatment ratio, included in the model as a time-dependent variable, is defined as the percentage of total endocrine treatment duration (AI+tamoxifen) spent on AI treatment. AI = Aromatase inhibitor, CI = Confidence interval, HER2 = Human Epidermal growth factor Receptor-2, HR = Hazard ratio, OS = Overall survival, PR = Progesterone receptor

c Interaction between the covariates and follow-up time centered at 5-years was included to accommodate non-proportional hazards. At 5-years of follow-up patients with 25%≤AI≤75% ratio had a smaller chance of an OS-event then patients with a AI<25% ratio (adjusted-HR 0.32). The HR increases by 42% for each additional year of follow-up, so at 6-years of follow-up the adjusted hazard ratio for 25%≤AI≤75% ratio vs AI<25% ratio = exp{ln(0.32)+(follow-up time-5)\*ln(1.42)}=0.45. At 5-years of follow-up patients with Grade II tumors had a smaller chance of an OS-event then patients with a Grade III tumor (adjusted-HR 0.55). The HR increases by 22% for each additional year of follow-up, so at 6-years of follow-up the adjusted hazard ratio for Grade II tumors vs Grade III tumors = exp{ln(0.55)+(follow-up time-5)\*ln(1.22)}=0.67.

**Figure Legends**

**Figure 1. Adjusted survival function of RFS for 2,284 ER+ breast cancer patients, according to AI-endocrine treatment ratio.** Adjusted 5-year RFS rates are 91.4% (95% CI = 90.2-94.7) vs 92.3% (95% CI = 90.6-95.3) vs 94.5% (95% CI = 93.0-96.8) for AI <25%, 25≤AI≤75%, and AI>75% respectively. The AI-endocrine treatment ratio, included in the model as a time-dependent variable, is defined as the percentage of total endocrine treatment duration (AI+tamoxifen) spent on AI treatment. The survival functions are obtained from a Cox model at average values of age at diagnosis, trastuzumab use (included as a time-dependent variable), grade, number of positive lymph nodes, pT-stage, PR status, HER2 status and ovarian ablation (included as a time-dependent variable). AI = Aromatase inhibitor, ER = Estrogen receptor, HER2 = Human Epidermal growth factor Receptor 2, PR = Progesterone receptor, pT-stage = pathologic T-stage, RFS=Recurrence-free survival

**Figure 2.** **Adjusted survival function of OS for 2,284 ER+ breast cancer patients, according to AI-endocrine treatment ratio.** Adjusted 5 year OS rates were 94.6% (95% CI = 93.6-96.1) vs 97.6% (95% CI = 97.0-98.4) vs 97.3% (95% CI = 96.4-98.4) for AI <25%, 25≤AI≤75% and AI>75% respectively. The AI-endocrine treatment ratio, included in the model as a time-dependent variable, is defined as the percentage of total endocrine treatment duration (AI+tamoxifen) spent on AI treatment. The survival functions are obtained from a Cox model at average values of age at diagnosis, trastuzumab use (included as a time-dependent variable), grade, number of positive lymph nodes, pT-stage, PR status, HER2 status and ovarian ablation (included as a time-dependent variable). AI= Aromatase inhibitor, ER=estrogen receptor, HER2 = Human Epidermal growth factor Receptor 2, OS = Overall survival, PR = Progesterone receptor, pT-stage = pathologic T-stage