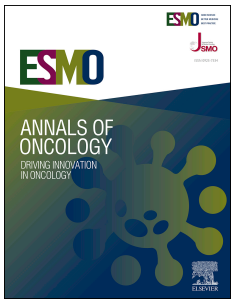

Impact of Anthracyclines in Genomic High Risk, Node-Negative, HR-Positive/HER2-Negative Breast Cancer

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PII: S0923-7534(25)00922-6

DOI: https://doi.org/10.1016/j.annonc.2025.08.002

Reference: ANNONC 1879

To appear in: *Annals of Oncology*

Received Date: 22 April 2025

Revised Date: 11 August 2025

Accepted Date: 13 August 2025

Please cite this article as: Chen N, Freeman J, Yarlagadda S, Atmakuri A, Kalinsky K, Pusztai L, Sparano J, Huo D, Nanda R, Howard F, Impact of Anthracyclines in Genomic High Risk, Node-Negative, HR-Positive/HER2-Negative Breast Cancer, *Annals of Oncology* (2025), doi: https://doi.org/10.1016/j.annonc.2025.08.002.

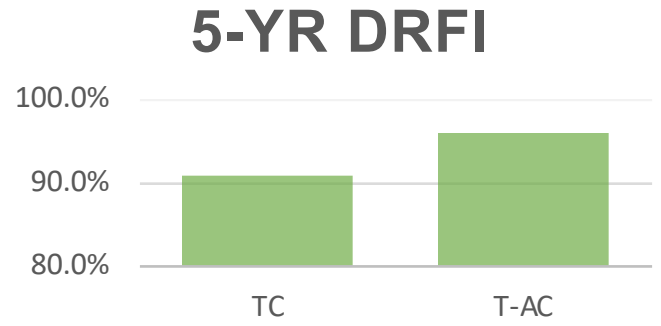
TAILORx post-hoc analysis demonstrates benefit in 5-yr recurrence and survival outcomes from the addition of anthracyclines to taxane-based regimen in early-stage, lymph-node negative HR+/HER2- breast cancers with 21-gene RS ≥ 31 and tumors ≥ 2cm

HR+/HER2-
Lymph node neg
RS ≥ 11

TC

T-AC

Alternative chemotherapy regimen or no chemotherapy



- In RS ≥ 31, significant benefit of 5-yr DRFI in pts receiving T-AC vs TC (96.1% vs 91%)
- In subgroup analysis, survival benefits and OS seen in pts also with tumors > 2cm
- Benefit of anthracyclines increases with increasing RS greater than 31

Original Research

Title: Impact of Anthracyclines in Genomic High Risk, Node-Negative, HR-Positive/HER2-Negative Breast Cancer

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Abstract

Background

The benefit of anthracyclines for patients with high 21-gene recurrence score (RS) is unclear, despite the widespread use of RS to guide adjuvant chemotherapy treatment for hormone receptor-positive (HR+)/HER2-negative (HER2-) breast cancer. This study aimed to assess whether patients with RS $\geq$ 31 would have improved outcomes with the addition of anthracyclines to taxane-based chemotherapy.

Patients and Methods

We included patients from TAILORx with RS ≥ 11 who received treatment with either taxanes with cyclophosphamide (TC) or taxane with anthracyclines/cyclophosphamide (T-AC). Distant recurrence-free interval (DRFI), distant recurrence-free survival (DRFS), overall survival (OS) were compared, controlling for age, tumor size and grade, receptor status, and RS. Spline regression was used to estimate adjusted hazard ratio (aHR) for receipt of T-AC (vs TC) for these endpoints as a function of RS.

Results

A total of 2,549 patients who received either T-AC or TC were included in the primary analysis. In patients with RS $\geq$ 31, receipt of T-AC was associated with improved DRFI (5-year rate of 96.1% with T-AC vs 91.0% with TC; aHR, 0.31; P = 0.006), DRFS (95.4% vs 89.8%; aHR, 0.49; P = 0.032), and a trend towards improved OS (adjusted 5-year rate 97.3% vs 93.6%; aHR, 0.67; P = 0.31). Spline regression demonstrated increasing anthracycline benefit with increasing RS.

Conclusion

Patients with early-stage, HR+/HER2- breast cancer with the highest genomic risk disease (RS $\geq$ 31) may benefit from the addition of an anthracycline to taxane-based adjuvant chemotherapy. Genomic RS testing may predict anthracycline benefit more accurately than clinicopathologic factors such as nodal status.

Keywords: breast cancer, HR+/HER2-negative, recurrence score, anthracyclines

Introduction

The role of chemotherapy in the adjuvant treatment of high-risk, early-stage hormone receptor-positive (HR+)/HER2-negative (HER2-) breast cancer has evolved. Cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) emerged as the first effective chemotherapy regimen for early-stage breast cancer,[1] and was subsequently supplanted by anthracycline-based,[2] followed by combination anthracycline and taxane chemotherapy.[3] However, given the side effect profile of anthracyclines, including cardiotoxicity and increased risk of secondary hematologic malignancies,[4,5] several clinical trials have evaluated the efficacy of anthracycline-containing compared to anthracycline-sparing regimens.

The Anthracyclines in Early Breast Cancer (ABC) trials evaluated the potential non-inferiority of adjuvant taxane plus cyclophosphamide (TC) for 6 cycles vs taxane plus anthracycline/cyclophosphamide (T-AC) with a primary endpoint of invasive disease-free survival (IDFS) in early-stage HER2-negative breast cancer.[6] In the overall population, with a hazard ratio (HR) of 1.23, non-inferiority was not demonstrated. However, in subgroup analysis of HR+ disease, while there was no benefit in IDFS in T-AC vs TC in node-negative patients, greater benefit was seen with increasing lymph nodes (LN) (HR, 0.69 in LN-; 1.14 in 1-3 LN; 1.46 in $\geq$ 4 LN).[6] An updated analysis with median of 6.9 years follow-up of the ABC trials found no benefit of anthracyclines in the overall subset of HR+ cases and a less clear relationship between number of LN and benefit of anthracyclines (HR, 0.95 in LN-; 1.12 in 1-3 LN; 1.06 in $\geq$ 4 LN).[7] Furthermore, other studies have failed to demonstrate a superiority of anthracycline plus taxane regimens to TC – including the Danish Breast Cancer Cooperative Group 07-READ trial and the West German Study PLAN B trial.[8,9] A large meta-analysis conducted by EBCTCG of twenty three randomized clinical trials evaluating taxane regimens with or without anthracyclines did not establish an increasing benefit of anthracyclines in patients with increasing clinical risk (HR, 0.50 in LN-; 0.69 in LN+) but rather found anthracyclines had a non-statistically significant benefit in early-stage HR+/HER2- cases, regardless of lymph node status (node negative and node positive).[10] Thus, an anthracycline-sparing regimen is often chosen for clinically lower-risk patients with HR+/HER2-, node-negative disease, whereas anthracyclines are frequently used in the treatment of higher stage HR+/HER2- and triple-negative breast cancer.[11,12]

However, it has since been established that only a fraction of HR+/HER2- breast cancer benefits from adjuvant chemotherapy; widespread use of genomic testing is now used to identify such cases. The TAILORx trial evaluated patients with node-negative, HR+/HER2- breast cancer with the 21 gene recurrence score (RS) and demonstrated no significant difference in recurrence-free survival (RFS) in patients with an intermediate RS of 11 – 25 in patients treated with endocrine therapy alone compared with endocrine therapy and chemotherapy.[13] Similar findings were seen in the RxPONDER trial of HR+/HER2- breast cancer with 1 – 3 lymph nodes involved, with no chemotherapy benefit in patients with an RS of $\leq$ 25[14] – although a benefit was seen in subsets of premenopausal patients in both trials, which might be due to the ovarian suppressive effects from chemotherapy.

Despite the widespread use of RS to guide the use of chemotherapy in general for patients with HR+/HER2- breast cancer with 0-3 positive nodes, it is uncertain if patients with higher RS scores benefit from more aggressive chemotherapy regimens incorporating anthracyclines. Although current guidelines support the use of chemotherapy when RS > 25, the initial 'high risk' cutoff of RS $\geq$ 31[15,16] may identify a subset with a more definitive chemotherapy benefit. The appropriate usage of anthracyclines in early-stage breast cancer remains controversial,[17-19] and biomarker-driven, individualized approaches are needed to decrease long-term cardiotoxicity and risk of secondary hematologic malignancies. This study aimed to assess whether tumors with a high RS ($\geq$ 31) may represent a smaller subset of chemo-sensitive tumors that demonstrate improved recurrence outcomes from the addition of anthracyclines to taxane-containing regimens. Outcomes for TC, T-AC, and other regimens are also presented.

Patients and Methods

Study Design and Data Source

We conducted a post-hoc analysis of patient-level data from the randomized phase III, international, TAILORx trial (NCT00310180, coordinated by the ECOG-ACRIN Cancer Research Group) which enrolled patients with stage I/II, node-negative, HR+/HER2- breast cancer.[13] De-identified, patient-level data was obtained from the National Clinical Trials Network/National Cancer Institute Community Oncology Research Program (NCTN/NCORP) data archive, representing the June 2022 data cutoff of TAILORx as

recently described.[20] This research was approved by the University of Chicago institutional review board (protocol 22-0707).

Study Population and Endpoints

In the TAILORx trial, patients with an RS between 11 and 25 were randomized to endocrine therapy or endocrine therapy plus chemotherapy of physicians' choice, whereas patients with an RS ≥ 26 received chemotherapy of physician's choice. We included patients with an RS ≥ 11 with known covariates of age, estrogen/progesterone receptor status, tumor size, and grade (**Supplemental Figure 1**). Estrogen and progesterone receptor positivity used for this study are from local pathology laboratory assessments, with no strict cutoff for expression defined per trial protocol. In this analysis, we included patients who received either no adjuvant chemotherapy, or chemotherapy that could be categorized as TC, T-AC, anthracycline without taxane (AC), and cyclophosphamide/methotrexate/fluorouracil (CMF) – as previously defined in publications of TAILORx.[21] Patients in the T-AC group could have received anthracycline plus cyclophosphamide (dose dense or standard) sequentially with taxane, concurrent anthracycline/cyclophosphamide/docetaxel, or other anthracycline and taxane-containing standard chemotherapy regimens. Patients in the AC group received standard or dose dense doxorubicin plus cyclophosphamide, epirubicin plus cyclophosphamide, or other anthracycline-based chemotherapy. Patients in the CMF group received oral or IV CMF. Menopausal status is defined as per the TAILORx protocol; post-menopausal patients included women ≥ 60 years of age, women 45-59 years of age with spontaneous cessation of menses > 12 months prior to registration or spontaneous cessation of menses < 12 months with follicle stimulating hormone (FSH) level in post-menopausal range, and women with prior bilateral oophorectomy.[13]

The primary endpoint of the study was distant recurrence-free interval (DRFI) in the RS ≥ 31 subgroup treated with TC vs T-AC. Secondary endpoints included recurrence-free interval (RFI), distant recurrence-free survival (DRFS), recurrence-free survival (RFS), disease-free survival (DFS), and overall survival (OS) compared between TC, T-AC, and other regimens. Clinicopathologic factors including patient

age, race, ethnicity, menopausal status, tumor size, tumor grade, estrogen/progesterone positivity and RS were also compared between patients receiving TC, T-AC, and other regimens.

Statistical Analysis

Demographic factors were compared across groups using ANOVA for continuous variables and Chi-squared tests for categorical variables. DRFI, RFI, DRFS, RFS, DFS, and OS as defined per TAILORx[13] and were compared using adjusted hazard ratios (aHR) controlling for age, tumor size, estrogen and progesterone receptor status, RS, treatment received, and interaction of treatment with high RS (using a cutoff of 31). Likelihood ratio test was used to determine interaction of treatment benefit with high RS (≥ 31) versus low RS (< 31). Kaplan-Meier estimates for 5-year event rates for these outcomes are also reported. Subgroup analyses across key clinicopathologic factors for outcome endpoints in the high RS were also evaluated in unadjusted Cox models to assess for heterogeneity of benefit. Restricted cubic spline regression was used to estimate aHR for receipt of T-AC, AC, CMF, or no chemotherapy (vs TC) for these endpoints as a function of RS; L2 regularization was used for spline estimates due to collinearity for some treatments. Finally, associations were also assessed between RSClin[22] predicted chemotherapy benefit (derived from age, tumor grade, tumor size, recurrence score, and endocrine therapy regimen) and survival endpoints. Mean imputation was used to estimate grade when unavailable for RSClin[22] calculation, and endocrine regimen was assigned as aromatase inhibitor for all patients given the high number of patients receiving a mixture of endocrine therapies. All statistical tests were 2-sided with a significance threshold of $P < 0.05$. All analyses were performed using Python 3.9.13 with the lifelines 0.28.0 package.

Results

Patient Characteristics

Of 7,789 cases that met study eligibility, 438 were treated with T-AC and 2,111 were treated with TC, 1,152 were treated with AC, 247 were treated with CMF, and 3,841 received no chemotherapy. Overall, the mean age was 55.3 years and the median follow-up time was 11.7 years for overall survival (**Table 1**). Patients treated with T-AC were younger (mean 53 vs 55 years old), more likely to be Hispanic (16% vs 7%), and

more likely to be premenopausal (42% vs 36%) compared to patients treated with TC. There were no significant differences in choice of chemotherapy regimen between racial groups. Patients treated with T-AC also had larger tumors (mean 20 mm vs 18 mm), were more likely to be high grade (36% vs 24%), were more likely estrogen receptor-negative (2.5% vs 1%) or progesterone receptor-negative (21% vs 14%), and had a higher RS (mean 30 vs 23) (**Table 1**). The average reported duration of endocrine therapy was 5.0 years in patients receiving T-AC and 5.3 years in patients receiving TC.

Patients with RS ≥ 31 Demonstrated Improved Recurrence Outcomes with Addition of Anthracyclines to Taxane Chemotherapy

In patients with an RS of ≥ 31, 306 received TC, 187 received AC, 173 received T-AC, 37 received no chemotherapy, and 22 received CMF. After adjusting for clinicopathologic covariates, receipt of T-AC was associated with improved outcomes at 5 years (**Figure 1** and **Table 2**). Evaluating the primary endpoint, the DRFI at 5 years was 96.1% with TAC compared to 91.0% with TC with RS ≥ 31 (aHR, 0.31; 95% CI, 0.14 to 0.72; *P* = 0.006; **Figure 1**). No difference was seen between T-AC and TC with RS < 31, with DRFI of 97.6% and 97.0% respectively at 5 years (aHR 1.42, 95% CI 0.88 – 2.29; *P* = 0.156, **Supplemental Figure 2**) – a significant interaction was seen between high RS and T-AC benefit (likelihood ratio *P* = 0.001). Similarly, there was an interaction between RS ≥ 31 and improved outcomes with T-AC for DRFS (aHR 0.49, 95% CI 0.26 – 0.94; *P* = 0.032; likelihood ratio *P* = 0.012), and a trend towards improved OS (aHR 0.67, 95% CI 0.31 – 1.44; *P* = 0.31; likelihood ratio *P* = 0.229) with T-AC in cases with RS ≥ 31 which was not statistically significant. Significant improvements in other recurrence endpoints (RFI, RFS, DFS) were also seen with T-AC with RS ≥ 31 (**Supplemental Table 1**). When analyzing other regimens, CMF was associated with reduced DRFI with RS ≥ 31 although an interaction test was not significant, perhaps due to the reduced efficacy of this regimen for lower recurrence scores as well (aHR, 2.99; 95% CI, 1.01 to 8.84, *P* = 0.047; interaction *P* = 0.16; **Supplemental Figure 3**).

Conversely, there was no difference in any endpoint between alternative treatment regimens and TC in the subset with RS < 31 (**Table 2**). Repeating the analysis to compare treatment efficacy in groups with RS 26 – 30 versus RS < 26, we found that no interaction between RS 26–30 and improvement in outcomes

with any specific chemotherapy regimen (**Supplemental Table 2**). The addition of anthracyclines to taxane chemotherapy can further increase the risk of ovarian insufficiency or failure.[23] Therefore, we also repeated our adjusted analysis in a Cox model for cases with high RS in subgroups of pre/postmenopausal patients (**Supplemental Table 3**). The benefit of anthracyclines in patients with RS ≥ 31 was similar in both groups – with, for example, a similar hazard ratio for DRFI with T-AC vs TC in pre-menopausal patients (aHR, 0.19; 95% CI, 0.04 to 0.79; $P = 0.022$) and postmenopausal (aHR, 0.25; 95% CI, 0.08 to 0.84; $P = 0.025$) patients. Further outcomes with T-AC vs TC in cases with RS ≥ 31 were analyzed in additional subgroups in unadjusted Cox models (**Supplemental Figure 4**), demonstrating consistent improved outcomes with T-AC except in T1 tumors where there was no signal of benefit. Finally, we assessed whether RSClin predicted chemotherapy benefit would more precisely distinguish patients benefiting from anthracyclines by integrating clinical and genomic factors (**Supplemental Table 4**). A cutoff of predicted chemotherapy benefit of 10% was chosen, corresponding to a similar proportion of the full study population (9.5%) as the RS ≥ 31 cutoff (9.8%). A trend towards improved 5-year DRFI with T-AC (94.2%) versus TC (90.9%) in patients with RSClin predicted chemotherapy benefit ≥ 10% was seen, but was not statistically significant (aHR 0.54, 95% CI 0.26 – 1.13, $P = 0.102$).

Estimation of Benefit of Specific Treatment Regimens Compared to TC as a Function of Recurrence Score

We performed a spline regression to estimate the benefit of the specified treatment regimens compared to TC on recurrence and survival outcomes as a function of RS. Spline regression estimated an increasing benefit of T-AC over TC with increasing RS (**Figure 2, Table 3**) – with an RS of 20, there was no significant benefit in T-AC for any survival measure. Conversely, with an RS of 60, there was a significant improvement in RFI (aHR, 0.35; 95% CI, 0.13 to 0.93) and DRFI (aHR, 0.34; 95% CI, 0.11 to 0.99) – with trends towards improvement in other outcome measures (**Supplemental Table 5**). There was no significant differences in comparison between TC and AC (**Supplemental Table 6**) in part due to small sample sizes, although AC trended towards worse outcomes with higher RS. Conversely, receipt of CMF (**Supplemental**

Table 7) or no chemotherapy (**Supplemental Table 8**) were associated with significantly worse outcomes with higher RS, although estimates were broad due to small sample size (**Supplemental Figure 5**).

Discussion

This post-hoc analysis of the TAILORx trial demonstrated a significant benefit in 5-year estimates of DRFI (96.1% vs 91.0%, adjusted HR 0.32, *P*=0.009), DRFS, RFI, RFS and a trend towards benefit in OS with the addition of anthracycline to taxane-containing chemotherapy in patients with RS ≥ 31. This benefit was statistically significant only when controlling for clinicopathologic factors including tumor size, tumor grade, and patient age. These adjustments are necessary as RS alone may not completely capture chemotherapy benefit and tools such as RSClin have been demonstrated to improve prediction of chemotherapy benefit by adjusting for clinical risk factors thus improving prediction of recurrence over RS alone.[20,22,24] Patterns of chemotherapy choice in this analysis were similar to other studies,[12] with patients of younger age, premenopausal status, larger tumors, and a higher RS being more likely to receive an anthracycline. In our analysis, all recurrence / survival outcomes – including overall survival – were improved in the subgroup of patients with RS ≥ 31 and tumor size > 2 cm, which may serve as a guideline to identify the small fraction of node negative patients who benefit from anthracyclines. Although other subgroups had trends towards improvement in recurrence metrics such as DRFI, this must be weighed against the risk of treatment-related toxicity – which may outweigh the potential benefits in smaller tumors. In long term follow-up (median of 6.9 years) of the ABC trials, addition of anthracyclines continued to be associated with reduction in recurrence but similar overall survival – with increased rates of leukemia and death unrelated to breast cancer in the anthracycline arm.[7] Additionally, given the approval of CDK4/6 inhibitors for this population of high risk T2N0 disease,[25] it will be important to determine if the use of improved adjuvant endocrine therapy can obviate the need for anthracycline-based therapy (and the associated cardiac and hematologic toxicities) in some of these high-risk patients.

Most studies evaluating the benefit of anthracyclines (such as those in the ABC trials) did not include subgroup analysis by genomic risk; subgroups were only categorized by clinical risk. The West

German Study PlanB Trial (included in the EBCTCG meta-analysis) randomized patients with RS > 11 to receive TC with or without epirubicin and overall did not show any benefit with the addition of an anthracycline.[8] The patient population with an RS > 25 comprised about 20% of the total, and subgroup analysis did not show benefit with the addition of epirubicin for this population.[8] Of note, epirubicin was used in PlanB, whereas doxorubicin was predominantly the anthracycline of choice in TAILORx and the ABC trials, which raises a possibility of differences in efficacy of these agents, although current data suggest equivalent outcomes with equimolar doses.[26,27] Similarly, a secondary analysis of the TAILORx study in patients with RS 26 – 100 categorized patients by chemotherapy regimen received, and did not find a significant difference in 5-year DRFI between T-AC and TC (95.1% [95% CI, 91 to 97.3] vs 92.7% [95% CI, 90 to 94.7]).[21] However, the lack of clear anthracycline benefit in these studies may be driven by patients with RS 26 – 30 who may not have derived as much benefit (as seen in our analysis). Furthermore, this unadjusted analysis did not control for prognostic covariates in patients with varying risk, and our adjusted analysis demonstrates a clearer benefit for anthracyclines in patients with higher genomic risk disease. Similarly, assessment of 21-gene RS to determine the benefit of the addition of paclitaxel to AC chemotherapy in NSABP B-28 similarly failed to demonstrate an association of paclitaxel benefit with RS, although the benefit of addition of paclitaxel was numerically highest in the subset with RS ≥ 26.[16]

While the 21-gene assay was designed to evaluate sensitivity to endocrine therapy[28] and has subsequently been used to identify patients who benefit from chemotherapy, this analysis suggests a benefit of more aggressive / anthracycline-containing chemotherapy with increasing RS above 31, thus providing evidence to potentially expand the scope of 21-gene assay as a predictive biomarker for benefit of specific chemotherapy regimens. Conversely, the RxPONDER study was designed to evaluate if increasing RS scores are associated with increasing chemotherapy benefit and did not find an association in a genomic low risk (RS ≤ 25) population.[14] This may be partially explained by the limited association of RS to tumor proliferation markers in low risk tumors.[28] Thus, the 21-gene assay may be better than current predictive biomarkers of anthracycline benefit such as clinical risk and nodal status. Indeed, we found that although RSClin is predictive of general chemotherapy benefit[24], RSClin estimates did not improve identification of patients requiring anthracyclines in our limited assessment. High predicted chemotherapy benefit (as

estimated by RSClin) can be due to both high genomic risk as well as other factors like large tumor size – the latter of which may indicate a higher recurrence risk but not a biologic sensitivity to anthracyclines.

RS 26 - 100 represents approximately 17% of HR+/HER2- breast cancers (of which 12% have RS $\geq$ 31 and 88% have RS 26-30) whereas the majority of patients with ER-low disease will have Oncotype scores of 26 or higher.[29] Thus, these findings are in line with current clinical practice – many of these higher RS cases may have weak hormone receptor expression and may have biology more akin to triple-negative disease, where the benefit of anthracyclines is more clear.[10] Oncotype is a composite gene expression score that incorporates the expression of the estrogen, progesterone, and HER2 receptors, proliferation and invasion genes, among others.[30] As the exact breakdown of gene expression is unavailable in this trial, we cannot conclude if one component of Oncotype conferred the greatest sensitivity to anthracycline therapy. However, it is notable that positive progesterone receptor expression was associated with a greater benefit from anthracyclines in our subgroup analysis (as seen in Supplemental Figure 4). This may suggest that cases that achieve a high Oncotype score due to weak estrogen / progesterone receptor expression benefit less from anthracyclines than those where the high score is the result of other factors, such as proliferation. Nonetheless, further work is needed to separate gene expression patterns associated with chemotherapy benefit from markers that are solely prognostic. Similar to the stratification of the 21-gene assay into high risk designations of ≥ 26 and ≥ 31, cases with high risk results from the MammaPrint 70-gene signature assay can be further stratified into High 1 and High 2, with the latter categorization having basal-like tumor properties and higher risk of recurrence.[31,32] A recent prospective, non-randomized analysis of patients undergoing MammaPrint testing demonstrated a decreased 3-year-RFI in patients with High 2 Luminal B-Type tumors treated with TC (86.4% vs 97.1%; $P = 0.0076$) as compared to T-AC.[33] This finding was not extended to High 1 tumors, suggesting the benefit of anthracyclines was limited to a higher risk patient population, even within the high risk category. With emerging next-generation prognostic tools utilizing clinical risk factors, genomics, and artificial intelligence, it will be important to assess if such approaches can similarly identify the highest risk patients who benefit from anthracycline therapy.[34-39]

There are limitations to this study. The TAILORx study was not powered to formally evaluate the benefit of anthracyclines in this setting, and there may be additional uncontrolled patient or disease specific

factors that led to selection of treatment regimen influencing results. This may include duration and compliance with chosen chemotherapy and endocrine therapy. Furthermore, the choice of chemotherapy was non-randomized and reflective of physician biases, resulting in a preference towards treatment with TC in this node-negative population. The benefit of T-AC compared to TC was only measurable when controlling for confounders such as the higher RS in patients receiving T-AC. TAILORx only enrolled node-negative disease, and thus small benefits of anthracyclines in lower risk patients (i.e., RS 26 – 30) may have been more difficult to measure due to the overall lower risk of disease recurrence in this population. Ultimately, patients with RS $\geq$ 31 are uncommon and represent a small portion of patients included in this overall analysis and in clinical practice. Although duration of endocrine therapy was similar across arms, data regarding compliance with adjuvant anti-estrogen therapy was not available and may have contributed to differences in outcomes.

Further evaluation of the benefit of anthracyclines utilizing the 21-gene RS in larger populations in the node-negative and node-positive populations is needed to better elucidate the role of anthracyclines in early-stage, HR+/HER2- breast cancer. Clinical risk and genomic risk should be considered together when evaluating benefit of additional anthracyclines. This data demonstrated that the benefit of anthracyclines in tumors with RS $\geq$ 31 was limited to patients with larger tumors. Nonetheless, anthracyclines may be considered in patients with the highest genomic risk HR+/HER2- node-negative breast cancer, but this must be carefully weighed against the risk of late anthracycline morbidity which may not be fully captured in this study.

Data Sharing Statement

The patient specific data from TAILORx analyzed in this study were obtained through the NCTN/NCORP Data Archive (nctn-data-archive.nci.nih.gov).

Acknowledgments

F.M.H. received support from the National Cancer Institute (K08CA283261), the Cancer Research Foundation, the Lynn Sage Breast Cancer Foundation, the Alliance for Clinical Trials in Oncology Foundation, and the Breast Cancer Research Foundation. D.H. and F.M.H. received support from the Department of Defense (BC211095, BC211095P1). J.Q.F. received support from the Susan G. Komen® Breast Cancer Foundation (TREND21675016) and the National Institute on Aging (T32AG000243). The contents are solely the responsibility of the authors and do not necessarily represent the official views of the National Cancer Institute and the National Institute on Aging. TAILORx was coordinated by the ECOG-ACRIN Cancer Research Group (Peter J. O'Dwyer, MD and Mitchell D. Schnall, MD, PhD, Group Co-Chairs) and supported by the National Cancer Institute of the National Institutes of Health under award numbers: U10CA180820, UG1CA233247, and UG1CA233337.

Authors' Disclosures of Potential Conflicts of Interest

NC reports consultant fees from Novartis, Guardant Health, Seagen, Stemline, AstraZeneca, Daiichi Sankyo and research funding from Eli Lilly, Olema, and Puma. JQF reports no relevant disclosures. SY reports no relevant disclosures. AA reports no relevant disclosures. KK reports consulting/advisory/speaker role for Daiichi Sankyo, Eli Lilly, Pfizer, Novartis, Eisai, AstraZeneca, Immunomedics, Merck, Seattle Genetics, Cyclacel, OncoSec, 4D Pharma, Puma, Genentech, Ascendant, Myovant, Takeda, and Menarini; and owns Grail stock options. LP reports has received consulting fees and honoraria for advisory board participation from Pfizer, Astra Zeneca, Merck, Novartis, Bristol-Myers Squibb, Stemline-Menarini, GlaxoSmithKline, Genentech/Roche, Personalis, Daiichi, Natera, Agendia, Exact Sciences, Radionetics, and institutional research funding from Seagen, GlaxoSmithKline, AstraZeneca, Merck, Pfizer and Bristol Myers Squibb. JS has received consulting fees from Genomic Health. DH reports no COI. RN reports consulting funding from

Astrazeneca, Daiichi Sankyo, Exact Sciences, GE, Gilead, Guardant Health, Merck, Moderna, Novartis, OBI, Pfizer, Sanofi, Seagen, Stemline, Summit Therapeutics and research funding from Arvinas, AstraZeneca, BMS, Corcept Therapeutics, Genentech/Roche, Gilead, GSK, Merck, Novartis, OBI Pharma, OncoSec, Pfizer, Relay, Seagen, Sun Pharma, Taiho. FMH reports consultant fees from Novartis and Leica Biosystems.

References:

1. Bonadonna G, Moliterni A, Zambetti M, et al. 30 years' follow up of randomised studies of adjuvant CMF in operable breast cancer: cohort study. *BMJ*. Jan 29 2005;330(7485):217. doi:10.1136/bmj.38314.622095.8F
2. Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet*. May 14-20 2005;365(9472):1687-717. doi:10.1016/s0140-6736(05)66544-0
3. Braybrooke J, Bradley R, Gray R, et al. Anthracycline-containing and taxane-containing chemotherapy for early-stage operable breast cancer: a patient-level meta-analysis of 100 000 women from 86 randomised trials. *The Lancet*. 2023/04/15/ 2023;401(10384):1277-1292. doi:https://doi.org/10.1016/S0140-6736(23)00285-4
4. Nishi M, Wang PY, Hwang PM. Cardiotoxicity of Cancer Treatments: Focus on Anthracycline Cardiomyopathy. *Arterioscler Thromb Vasc Biol*. Nov 2021;41(11):2648-2660. doi:10.1161/ATVBAHA.121.316697
5. Balduzzi A, Castiglione-Gertsch M. Leukemia risk after adjuvant treatment of early breast cancer. *Womens Health (Lond)*. Jul 2005;1(1):73-85. doi:10.2217/17455057.1.1.73
6. Blum JL, Flynn PJ, Yothers G, et al. Anthracyclines in Early Breast Cancer: The ABC Trials-USOR 06-090, NSABP B-46-I/USOR 07132, and NSABP B-49 (NRG Oncology). *J Clin Oncol*. Aug 10 2017;35(23):2647-2655. doi:10.1200/JCO.2016.71.4147
7. Geyer CE, Jr., Blum JL, Yothers G, et al. Long-Term Follow-Up of the Anthracyclines in Early Breast Cancer Trials (USOR 06-090, NSABP B-46-I/USOR 07132, and NSABP B-49 [NRG Oncology]). *J Clin Oncol*. Apr 20 2024;42(12):1344-1349. doi:10.1200/JCO.23.01428
8. Nitz U, Gluz O, Clemens M, et al. West German Study PlanB Trial: Adjuvant Four Cycles of Epirubicin and Cyclophosphamide Plus Docetaxel Versus Six Cycles of Docetaxel and Cyclophosphamide in HER2-Negative Early Breast Cancer. *Journal of Clinical Oncology*. 2019;37(10):799-808. doi:10.1200/jco.18.00028
9. Ejlertsen B, Tuxen MK, Jakobsen EH, et al. Adjuvant Cyclophosphamide and Docetaxel With or Without Epirubicin for Early TOP2A-Normal Breast Cancer: DBCG 07-READ, an Open-Label, Phase III, Randomized Trial. *J Clin Oncol*. Aug 10 2017;35(23):2639-2646. doi:10.1200/jco.2017.72.3494
10. Early Breast Cancer Trialists' Collaborative Group . Electronic address bocoau, Early Breast Cancer Trialists' Collaborative G. Anthracycline-containing and taxane-containing chemotherapy for early-stage operable breast cancer: a patient-level meta-analysis of 100 000 women from 86 randomised trials. *Lancet*. Apr 15 2023;401(10384):1277-1292. doi:10.1016/S0140-6736(23)00285-4
11. O'Rourke J, Warnick J, Doole J, et al. Exploring Breast Cancer Systemic Drug Therapy Patterns in Real-World Data. *JCO Clin Cancer Inform*. Sep 2023;7:e2300061. doi:10.1200/cci.23.00061
12. Henderson J, Adams P, Barber K. Factors Determining Anthracycline Use in Hormone Receptor Positive, Early-Stage Breast Cancer. *Clinical Breast Cancer*. 2019/06/01/ 2019;19(3):e475-e480. doi:https://doi.org/10.1016/j.clbc.2019.01.012
13. Sparano JA, Gray RJ, Makower DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. *N Engl J Med*. Jul 12 2018;379(2):111-121. doi:10.1056/NEJMoa1804710
14. Kalinsky K, Barlow WE, Gralow JR, et al. 21-Gene Assay to Inform Chemotherapy Benefit in Node-Positive Breast Cancer. *N Engl J Med*. Dec 16 2021;385(25):2336-2347. doi:10.1056/NEJMoa2108873
15. Albain KS, Barlow WE, Shak S, et al. Prognostic and predictive value of the 21-gene recurrence score assay in postmenopausal women with node-positive, oestrogen-receptor-positive breast cancer on chemotherapy: a retrospective analysis of a randomised trial. *Lancet Oncol*. Jan 2010;11(1):55-65. doi:10.1016/S1470-2045(09)70314-6
16. Mamounas EP, Tang G, Paik S, et al. 21-Gene Recurrence Score for prognosis and prediction of taxane benefit after adjuvant chemotherapy plus endocrine therapy: results from NSABP B-28/NRG Oncology. *Breast Cancer Res Treat*. Feb 2018;168(1):69-77. doi:10.1007/s10549-017-4550-8
17. Piccart M, Nader-Marta G. Abstract Db1-01: Anthracyclines …to give or not to give? "For" argument. *Cancer Research*. 2024;84(9_Supplement):Db1-01-Db1-01. doi:10.1158/1538-7445.SABCS23-Db1-01
18. Vuger AT, Tiscoski K, Apolinario T, Cardoso F. Anthracyclines in the treatment of early breast cancer friend or foe? *Breast*. Oct 2022;65:67-76. doi:10.1016/j.breast.2022.06.007
19. Hurvitz SA, McAndrew NP, Bardia A, et al. A careful reassessment of anthracycline use in curable breast cancer. *NPJ Breast Cancer*. Oct 8 2021;7(1):134. doi:10.1038/s41523-021-00342-5
20. Sparano JA, Crager M, Gray RJ, et al. Clinical and Genomic Risk for Late Breast Cancer Recurrence and Survival. *NEJM Evidence*. 2024;3(8):EVIDoa2300267. doi:doi:10.1056/EVIDoa2300267

21. Sparano JA, Gray RJ, Makower DF, et al. Clinical Outcomes in Early Breast Cancer With a High 21-Gene Recurrence Score of 26 to 100 Assigned to Adjuvant Chemotherapy Plus Endocrine Therapy: A Secondary Analysis of the TAILORx Randomized Clinical Trial. *JAMA Oncology*. 2020;6(3):367-374. doi:10.1001/jamaoncol.2019.4794
22. Sparano JA, Crager MR, Tang G, Gray RJ, Stemmer SM, Shak S. Development and Validation of a Tool Integrating the 21-Gene Recurrence Score and Clinical-Pathological Features to Individualize Prognosis and Prediction of Chemotherapy Benefit in Early Breast Cancer. *Journal of Clinical Oncology*. 2021;39(6):557-564. doi:10.1200/jco.20.03007
23. Mauri D, Gazouli I, Zarkavelis G, et al. Chemotherapy Associated Ovarian Failure. *Front Endocrinol (Lausanne)*. 2020;11:572388. doi:10.3389/fendo.2020.572388
24. Vannier AGL, Dhungana A, Zhao F, et al. Validation of the RSClin risk calculator in the National Cancer Data Base. *Cancer*. Apr 15 2024;130(8):1210-1220. doi:10.1002/cncr.35163
25. Slamon D, Lipatov O, Nowecki Z, et al. Ribociclib plus Endocrine Therapy in Early Breast Cancer. *New England Journal of Medicine*. 2024;390(12):1080-1091. doi:doi:10.1056/NEJMoa2305488
26. Khasraw M, Bell R, Dang C. Epirubicin: is it like doxorubicin in breast cancer? A clinical review. *Breast*. Apr 2012;21(2):142-9. doi:10.1016/j.breast.2011.12.012
27. Kaklamani VG, Gradishar WJ. Epirubicin versus doxorubicin: which is the anthracycline of choice for the treatment of breast cancer? *Clin Breast Cancer*. Apr 2003;4 Suppl 1:S26-33. doi:10.3816/cbc.2003.s.012
28. Buus R, Sestak I, Kronenwett R, et al. Molecular Drivers of Oncotype DX, Prosigna, EndoPredict, and the Breast Cancer Index: A TransATAC Study. *J Clin Oncol*. Jan 10 2021;39(2):126-135. doi:10.1200/jco.20.00853
29. Higgins T, Kantor O, Harrison B, et al. Defining the Biology of Estrogen Receptor-Low-Positive Breast Cancer. *Annals of Surgical Oncology*. 2024/04/01 2024;31(4):2244-2252. doi:10.1245/s10434-023-14835-z
30. Paik S, Shak S, Tang G, et al. A Multigene Assay to Predict Recurrence of Tamoxifen-Treated, Node-Negative Breast Cancer. *New England Journal of Medicine*. 2004;351(27):2817-2826. doi:doi:10.1056/NEJMoa041588
31. Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. *N Engl J Med*. Aug 25 2016;375(8):717-29. doi:10.1056/NEJMoa1602253
32. Wolf DM, Yau C, Wulfkuhle J, et al. Redefining breast cancer subtypes to guide treatment prioritization and maximize response: Predictive biomarkers across 10 cancer therapies. *Cancer Cell*. Jun 13 2022;40(6):609-623.e6. doi:10.1016/j.ccell.2022.05.005
33. O'Shaughnessy J, Graham CL, Whitworth P, et al. Association of MammaPrint index and 3-year outcome of patients with HR+HER2- early-stage breast cancer treated with chemotherapy with or without anthracycline. *Journal of Clinical Oncology*. 2024;42(16_suppl):511-511. doi:10.1200/JCO.2024.42.16_suppl.511
34. Howard FM, Dolezal J, Kochanny S, et al. Integration of clinical features and deep learning on pathology for the prediction of breast cancer recurrence assays and risk of recurrence. *npj Breast Cancer*. 2023/04/14 2023;9(1):25. doi:10.1038/s41523-023-00530-5
35. Amgad M, Hodge JM, Elsebaie MAT, et al. A population-level digital histologic biomarker for enhanced prognosis of invasive breast cancer. *Nature Medicine*. 2024/01/01 2024;30(1):85-97. doi:10.1038/s41591-023-02643-7
36. Dhungana A, Vannier A, Zhao F, et al. Development and validation of a clinical breast cancer tool for accurate prediction of recurrence. *npj Breast Cancer*. 2024/06/15 2024;10(1):46. doi:10.1038/s41523-024-00651-5
37. Cho SY, Lee JH, Ryu JM, et al. Deep learning from HE slides predicts the clinical benefit from adjuvant chemotherapy in hormone receptor-positive breast cancer patients. *Scientific Reports*. 2021/08/30 2021;11(1):17363. doi:10.1038/s41598-021-96855-x
38. Cohen S, Shamai G, Sabo E, et al. Prediction of OncotypeDX recurrence score using H&E stained WSI images. *medRxiv*. 2025:2025.07.21.25331907. doi:10.1101/2025.07.21.25331907
39. Shamai G, Cohen S, Binenbaum Y, et al. Deep Learning on Histopathological Images to Predict Breast Cancer Recurrence Risk and Chemotherapy Benefit. *medRxiv*. 2025:2025.05.15.25327686. doi:10.1101/2025.05.15.25327686

Table 1: Baseline Demographic and Clinical Characteristics of the Study Population

		Regimen Group					*P*
Characteristic		**T-AC**	**TC**	**AC**	**CMF**	**None**	
	n	438	2111	1152	247	3841	
Age, n (%)	< 50	152 (34.7)	621 (29.4)	338 (29.3)	56 (22.7)	1057 (27.5)	0.004
	≥ 50	286 (65.3)	1490 (70.6)	814 (70.7)	191 (77.3)	2784 (72.5)	
Race, n (%)	Asian	14 (3.2)	80 (3.8)	48 (4.2)	14 (5.7)	157 (4.1)	0.083
	Black	33 (7.5)	171 (8.1)	63 (5.5)	21 (8.5)	282 (7.3)	
	Native Hawaiian or Pacific Islander	2 (0.5)	4 (0.2)	4 (0.3)	1 (0.4)	10 (0.3)	
	Not reported	18 (4.1)	96 (4.5)	27 (2.3)	11 (4.5)	118 (3.1)	
	White	371 (84.7)	1749 (82.9)	1004 (87.2)	200 (81.0)	3252 (84.7)	
	Multiracial	--	3 (0.1)	--	--	5 (0.1)	
	Native American	--	8 (0.4)	6 (0.5)	--	17 (0.4)	
Ethnicity, n (%)	Not Hispanic	304 (69.4)	1687 (79.9)	866 (75.2)	184 (74.5)	3151 (82.0)	<0.001
	Hispanic	69 (15.8)	151 (7.2)	162 (14.1)	33 (13.4)	300 (7.8)	
	Not reported	65 (14.8)	273 (12.9)	124 (10.8)	30 (12.1)	390 (10.2)	
Menopausal Status, n (%)	Premenopausal	182 (41.6)	752 (35.6)	416 (36.1)	66 (26.7)	1301 (33.9)	0.001
	Postmenopausal	256 (58.4)	1359 (64.4)	736 (63.9)	181 (73.3)	2540 (66.1)	
Tumor Size, mean (SD)		19.6 (9.0)	17.7 (8.1)	17.6 (9.6)	16.6 (7.9)	16.9 (8.1)	<0.001
Grade, n (%)	Low	63 (14.4)	461 (21.8)	236 (20.5)	40 (16.2)	1117 (29.1)	<0.001
	Med	203 (46.3)	1096 (51.9)	622 (54.0)	138 (55.9)	2107 (54.9)	
	High	159 (36.3)	504 (23.9)	263 (22.8)	51 (20.6)	492 (12.8)	
	Not Reported	13 (3.0)	50 (2.4)	31 (2.7)	18 (7.3)	125 (3.3)	
ER status, n (%)	Positive	427 (97.5)	2092 (99.1)	1142 (99.1)	246 (99.6)	3835 (99.8)	<0.001
	Negative	11 (2.5)	19 (0.9)	10 (0.9)	1 (0.4)	6 (0.2)	
PR status, n (%)	Positive	348 (79.5)	1810 (85.7)	1006 (87.3)	214 (86.6)	3522 (91.7)	<0.001
	Negative	90 (20.5)	301 (14.3)	146 (12.7)	33 (13.4)	319 (8.3)	
Recurrence Score, n (%)	11-25	196 (44.7)	1554 (73.6)	820 (71.2)	195 (78.9)	3752 (97.7)	<0.001
	26-30	69 (15.8)	251 (11.9)	145 (12.6)	30 (12.1)	52 (1.4)	
	31-100	173 (39.5)	306 (14.5)	187 (16.2)	22 (8.9)	37 (1.0)	
	Dose dense T-AC	186 (42.5)	--	--	--	--	<0.001

Chemotherapy Regimen Received, n (%)	Other anthracycline and taxane	85 (19.4)	--	--	--	--	
	Standard T-AC	110 (25.1)	--	--	--	--	
	Concurrent TAC*	57 (13.0)	--	--	--	--	
	TC	--	2111 (100.0)	--	--	--	
	Dose dense AC	--	--	284 (24.7)	--	--	
	Standard AC	--	--	749 (65.0)	--	--	
	Standard FEC	--	--	92 (8.0)	--	--	
	Other anthracycline without taxane	--	--	27 (2.3)	--	--	
	IV CMF	--	--	--	216 (87.4)	--	
	Oral CMF	--	--	--	31 (12.6)	--	
	None	--	--	--	--	3841 (100.0)	
Adjuvant Endocrine Therapy Received, n (%)	AI	196 (44.7)	1031 (48.8)	531 (46.1)	141 (57.1)	1849 (48.1)	<0.001
	OFS	14 (3.2)	14 (0.7)	20 (1.7)	--	65 (1.7)	
	OFS & AI	14 (3.2)	53 (2.5)	34 (3.0)	4 (1.6)	136 (3.5)	
	Tamoxifen	81 (18.5)	397 (18.8)	223 (19.4)	36 (14.6)	797 (20.7)	
	Sequential Tamoxifen & AI	117 (26.7)	587 (27.8)	325 (28.2)	59 (23.9)	919 (23.9)	
	Other	--	1 (0.0)	2 (0.2)	--	7 (0.2)	
	Not Reported	16 (3.7)	28 (1.3)	17 (1.5)	7 (2.8)	68 (1.8)	
Duration of Endocrine Therapy, mean years (SD)		5.0 (2.1)	5.3 (2.0)	5.4 (2.0)	5.6 (2.1)	5.3 (2.1)	0.005

Abbreviations: T-AC, Taxane and anthracycline chemotherapy; TC, docetaxel and cyclophosphamide; AC-T, anthracycline and cyclophosphamide followed by taxane; CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; AI, aromatase inhibitor; OFS, ovarian function suppression; ER, estrogen receptor; PR, progesterone receptor; FEC, fluorouracil, epirubicin hydrochloride, and cyclophosphamide.
* Note: concurrent TAC refers to docetaxel, doxorubicin, and cyclophosphamide given simultaneously

Table 2. Distant Recurrence and Survival Outcomes as a Function of Regimen and 21-Gene Recurrence Score Group

	RS	Treatment	Distant Recurrence-Free Interval	Distant Recurrence-Free Survival	Overall Survival
5 Year Event Rate (95% CI)	<31	TC	97.6 (96.7 - 98.2)	96.1 (95.0 - 96.9)	98.3 (97.6 - 98.8)
		T-AC	97.0 (93.9 - 98.6)	95.0 (91.4 - 97.2)	97.6 (94.6 - 98.9)
		AC	98.0 (96.9 - 98.8)	95.6 (94.1 - 96.8)	97.7 (96.6 - 98.5)
		CMF	98.1 (95.0 - 99.3)	96.6 (93.0 - 98.4)	98.6 (95.7 - 99.5)
		None	98.0 (97.5 - 98.4)	95.8 (95.1 - 96.4)	98.0 (97.5 - 98.4)
	≥31	TC	91.0 (86.8 - 94.0)	89.3 (84.8 - 92.6)	93.6 (89.9 - 96.0)
		T-AC	96.1 (91.5 - 98.2)	95.5 (90.7 - 97.8)	97.3 (93.0 - 99.0)
		AC	90.6 (84.8 - 94.2)	89.9 (84.0 - 93.7)	95.3 (90.8 - 97.6)
		CMF	79.8 (54.7 - 91.9)	79.8 (54.7 - 91.9)	83.6 (57.3 - 94.4)
		None	89.8 (71.7 - 96.6)	82.9 (63.5 - 92.5)	84.5 (63.5 - 93.9)
Adjusted HR (95% CI), p	<31	TC	ref	ref	ref
		T-AC	1.42 (0.88 - 2.29), 0.156	1.24 (0.85 - 1.81), 0.256	1.15 (0.73 - 1.80), 0.551
		AC	0.95 (0.68 - 1.32), 0.754	1.07 (0.85 - 1.35), 0.569	1.08 (0.83 - 1.40), 0.573
		CMF	1.26 (0.73 - 2.17), 0.414	1.08 (0.73 - 1.62), 0.694	1.00 (0.64 - 1.56), 0.995
		None	1.05 (0.82 - 1.33), 0.709	1.12 (0.94 - 1.33), 0.203	1.11 (0.91 - 1.34), 0.309
	≥31	TC	ref	ref	ref
		T-AC	**0.31 (0.14 - 0.72), 0.006**	**0.49 (0.26 - 0.94), 0.032**	0.67 (0.31 - 1.44), 0.308
		AC	1.30 (0.76 - 2.22), 0.346	1.07 (0.67 - 1.72), 0.767	1.02 (0.56 - 1.84), 0.954
		CMF	**2.99 (1.01 - 8.84), 0.047**	1.56 (0.60 - 4.03), 0.357	1.41 (0.48 - 4.15), 0.528
		None	1.32 (0.40 - 4.33), 0.652	1.65 (0.65 - 4.19), 0.291	1.96 (0.68 - 5.62), 0.209
RS ≥ 31 x Regimen Interaction HR (95% CI), Likelihood ratio p		T-AC	**0.22 (0.08 - 0.58), 0.001**	**0.40 (0.19 - 0.84), 0.012**	0.59 (0.24 - 1.42), 0.229
		AC	1.37 (0.73 - 2.58), 0.335	1.00 (0.59 - 1.70), 0.989	0.94 (0.50 - 1.80), 0.860
		CMF	2.38 (0.72 - 7.95), 0.184	1.44 (0.52 - 4.01), 0.499	1.41 (0.44 - 4.49), 0.570
		None	1.26 (0.37 - 4.23), 0.720	1.48 (0.57 - 3.81), 0.439	1.78 (0.61 - 5.18), 0.325

Abbreviations: T-AC, Taxane and anthracycline chemotherapy; TC, docetaxel and cyclophosphamide; AC, anthracycline chemotherapy (without taxane); CMF, cyclophosphamide, methotrexate, and 5-fluorouracil; HR, hazard ratio; CI:, confidence interval.

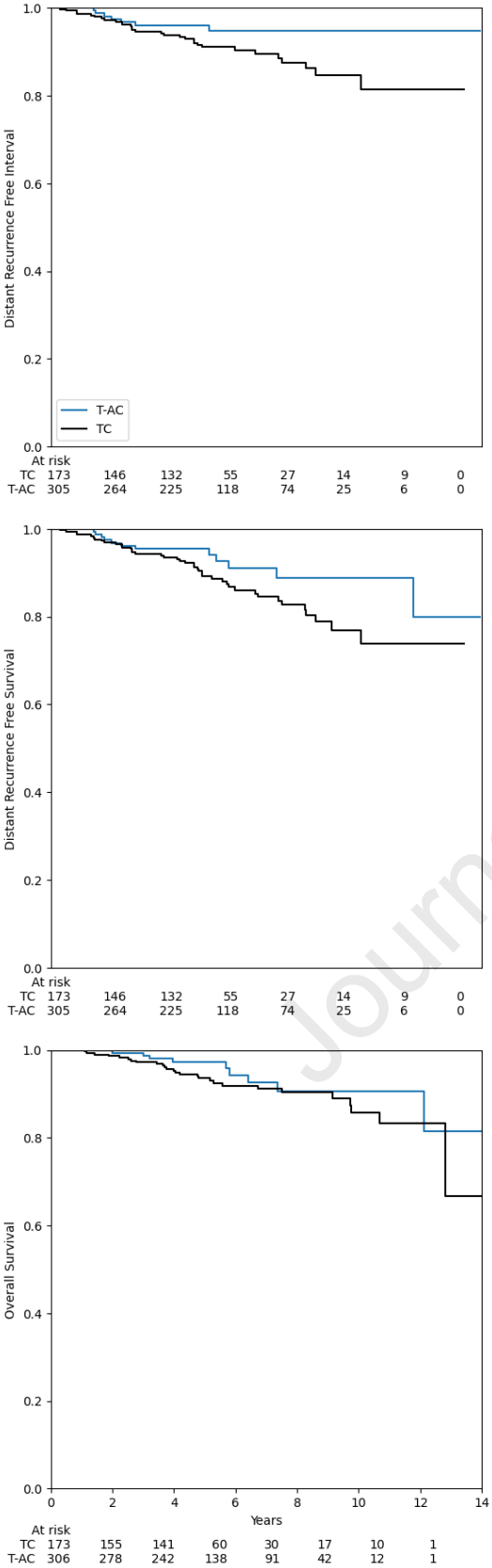
Adjusted hazard ratios are calculated with a multivariable Cox model including age, tumor size, estrogen / progesterone receptor status, recurrence score, treatment and interaction of treatment with high recurrence score for each treatment versus TC. Interaction p-values are per log likelihood test comparing model with / without interaction term.

Table 3. Estimated Distant Recurrence and Survival Benefit of Taxane and Anthracycline Chemotherapy as a Function of 21-Gene Recurrence Score

RS	Distant Recurrence-Free Interval	Distant Recurrence-Free Survival	Overall Survival
15	1.17 (0.67 - 2.04)	1.06 (0.69 - 1.62)	1.06 (0.66 - 1.72)
20	1.13 (0.69 - 1.86)	1.06 (0.73 - 1.56)	1.06 (0.68 - 1.66)
25	1.04 (0.63 - 1.72)	1.03 (0.68 - 1.56)	1.03 (0.64 - 1.67)
30	0.92 (0.56 - 1.50)	0.97 (0.64 - 1.46)	0.98 (0.61 - 1.59)
35	0.78 (0.48 - 1.27)	0.89 (0.59 - 1.33)	0.93 (0.57 - 1.50)
40	0.66 (0.39 - 1.12)	0.81 (0.52 - 1.25)	0.86 (0.51 - 1.47)
45	0.56 (0.30 - 1.05)	0.73 (0.44 - 1.23)	0.81 (0.43 - 1.51)
50	0.47 (0.22 - 1.01)	0.67 (0.35 - 1.25)	0.76 (0.36 - 1.60)

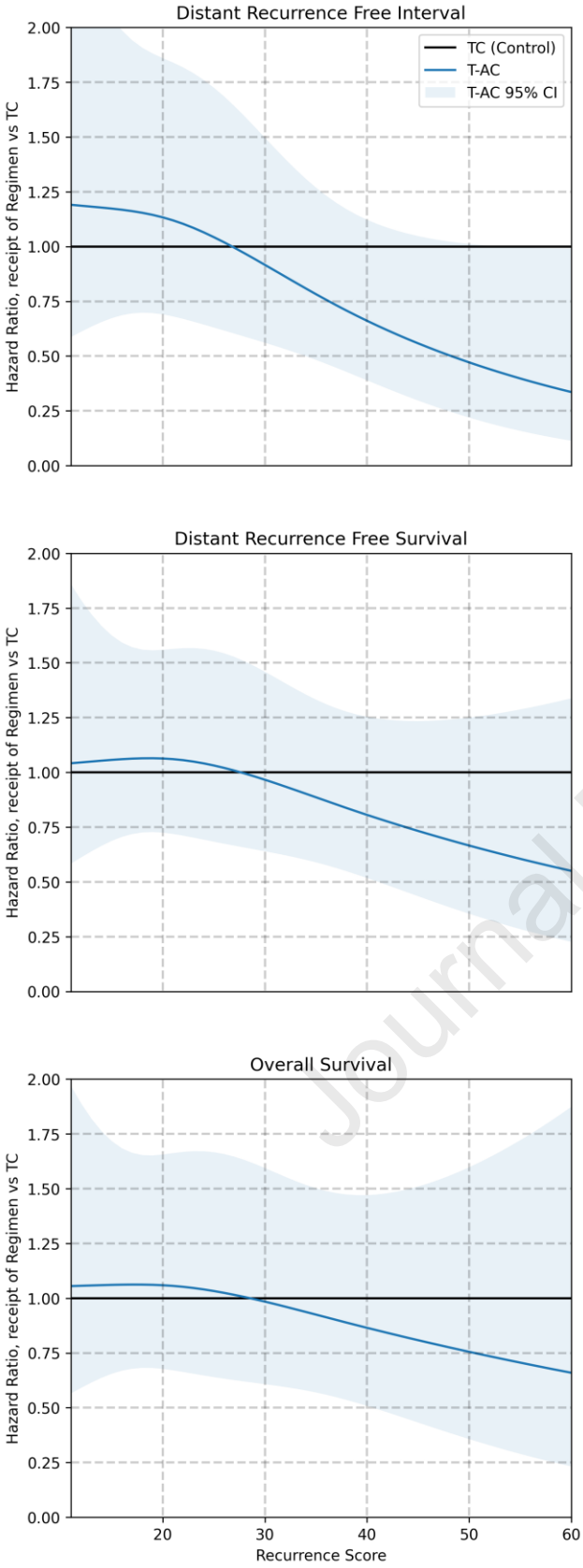
Hazard ratios were estimated for taxane and anthracycline chemotherapy compared to docetaxel and cyclophosphamide as a function of recurrence score (RS) using a restricted cubic spline with three knots; the model was additionally adjusted for age, tumor size, and estrogen / progesterone receptor status. Listed are adjusted hazard ratios with 95% confidence intervals for each endpoint at each recurrence score cutoff.

Figure 1. Distant Recurrence and Survival Outcomes with Taxane and Anthracycline Chemotherapy for Patients with 21-Gene Recurrence Score of 31 or Higher



Abbreviations: T-AC, Taxane and anthracycline chemotherapy; TC, docetaxel and cyclophosphamide.

Figure 2. Spline Regression of Treatment with Anthracycline with Increasing Recurrence Score



Abbreviations: T-AC, Taxane and anthracycline chemotherapy; TC, docetaxel and cyclophosphamide; CI, confidence interval.

Hazard ratios were estimated for the listed regimens compared to TC as a function of recurrence score using a restricted cubic spline with three knots. All models were additionally adjusted for age, tumor size, and estrogen / progesterone receptor status.