Adjuvant Dose-Dense Chemotherapy in Hormone Receptor–Positive Breast Cancer

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ABSTRACT

PURPOSE In light of evolving evidence that some patients with node-positive estrogen receptor–positive (ER+) disease may receive less benefit from chemotherapy, this study reports 12-year outcomes of the C9741 trial overall, and by the sensitivity to endocrine therapy (SET2,3) test index, a biomarker measuring endocrine transcriptional activity, to identify patients most likely to benefit from dose-dense chemotherapy.

METHODS In all, 1,973 patients were randomly assigned to dose-dense versus conventional chemotherapy. Hazard ratios (HRs) for prognosis and for predictive interaction with chemotherapy schedule were estimated from Cox models of long-term disease-free survival (DFS) and overall survival (OS). SET2,3 was tested on the 682 banked RNA samples from ER+ cancers.

RESULTS Dose-dense chemotherapy improved DFS in the overall study population by 23% (HR, 0.77 [95% CI, 0.66 to 0.90]) and OS by 20% (HR, 0.80 [95% CI, 0.67 to 0.95]); the benefits of dose-dense therapy were seen for ER+ and ER-negative subsets, without significant interaction between treatment arm and ER status. Low SET2,3 status was highly prognostic, but also predicted improved outcomes from dose-dense chemotherapy (interaction $P = .0998$ for DFS; 0.027 for OS), independent of menopausal status. Specifically, low endocrine transcriptional activity predicted benefit from dose-dense chemotherapy, whereas tumor burden and proliferation-driven signatures for molecular subtype classification did not.

CONCLUSION At 12-year follow-up, C9741 confirmed the sustained long-term benefit of adjuvant dose-dense chemotherapy for node-positive breast cancer. SET2,3 identified patients with ER+ breast cancer who benefited from dose-dense chemotherapy, and specifically, this benefit was predicted by low endocrine activity in the cancer, rather than tumor burden, molecular subtype, or menopausal status.

ACCOMPANYING CONTENT

Data Sharing Statement

Data Supplement

Protocol

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INTRODUCTION

The CALBG (Alliance) C9741 phase III trial established dose-dense scheduling of adjuvant chemotherapy as a standard of care for patients with node-positive breast cancer. Using a factorial design, C9741 randomized treatment with doxorubicin (A), cyclophosphamide (C), and paclitaxel (T) according to (1) a dose every 2 weeks (dose-dense) versus every 3 weeks (conventional) and (2) sequential (A→T→C) versus concurrent (AC→T) chemotherapy.[1] After a median follow-up of 3 years, dose-dense administration was associated with a 26% and 31% relative risk reduction for disease-free survival (DFS) and overall survival (OS), respectively.[1] The survival benefits in favor of dose-dense chemotherapy were seen irrespective of nodal, menopausal, and estrogen receptor (ER) status.[1] However, after 6 years of follow-up, an analysis described a significant interaction between ER status and dose-dense chemotherapy, favoring the dose-dense regimen only in patients with ER-negative (ER−) breast cancer.[2]

Concerns related to long-term anthracycline toxicity led to widespread use of nonanthracycline regimens such as four to six cycles of docetaxel and cyclophosphamide, particularly for patients with ER-positive (ER+) breast cancer or patients less able to tolerate AC→T chemotherapy. However, in 2019, the Oxford Overview meta-analysis of 26 clinical trials confirmed that dose-dense therapy reduced breast cancer recurrence and mortality.[3] Those benefits were similar for pre- and postmenopausal women.[3]

CONTEXT

Key Objective

To determine whether the long-term outcomes from the CALGB 9741 trial demonstrate a benefit from dose-dense chemotherapy in patients with estrogen receptor–positive (ER+) breast cancer.

Knowledge Generated

Patients with ER+ breast cancer had 20% improvement in disease-free survival from dose-dense chemotherapy treatments. The benefit was predicted by low level of endocrine transcriptional activity in ER+ breast cancer, not by proliferation-related molecular subtype or burden of disease.

Relevance *(G. Fleming)*

Dose dense adjuvant therapy provides long term benefits for a subset of patients with ER positive breast cancer. Continued investigation to reliably identify that subset is warranted.*

*Relevance section written by *JCO* Associate Editor Gini Fleming, MD.

Furthermore, a recent Oxford meta-analysis of 86 trials demonstrated significant reductions in breast cancer recurrence and mortality with combined taxane and anthracycline–based regimens.[4] And yet, omission of adjuvant chemotherapy appears to be safe for patients with low-risk ER+ breast cancer by genomic test who are either node-negative or have limited nodal involvement in the postmenopausal setting, whereas premenopausal patients seemed to benefit from adjuvant chemotherapy regardless of prognostic genomic assay result.[5-7] In C9741, after 12 years of follow-up, an analysis using the PAM50 intrinsic subtype method found that the assay was prognostic, but not predictive of benefit of dose-dense therapy in the overall study population.[8] So controversy surrounding the use and selection of adjuvant chemotherapy for ER+ breast cancer relates to opposing inferences from contemporary clinical trials and the meta-analyses of older clinical trials, and modern genomic testing has not proved as helpful in therapeutic decision making.

In the current study, we report DFS and OS from C9741 at 12 years of median follow-up evaluating the benefits of dose-dense versus conventional regimen across clinically relevant subsets with a focus on ER+ disease. Given that the efficacy of dose-dense chemotherapy relies on the premise that cancer cells, if not fully recovered during treatment interval, become more susceptible to subsequent doses,[9] and endocrine activity indicates cellular differentiation and survival, we hypothesized that dose-dense chemotherapy would be more effective in the subset of tumors with low endocrine transcriptional activity. To test this hypothesis, we used the sensitivity to endocrine therapy (SET2,3) test, a genomic test designed to measure the endocrine transcriptional activity ($SET_{ER/PR}$) in breast cancer, adjusted by a baseline prognostic index (BPI) that combines measures of tumor burden and molecular subtype.[10-14]

METHODS

Study Population and End Points

C9741 enrolled women after surgery for node-positive adenocarcinoma of the breast.[1] The primary study end point was DFS, measured from study entry until local recurrence, distant relapse, or death without relapse, whichever occurred first. The secondary end point was OS, measured from study entry until death from any cause. Disease-free and surviving patients were censored at the date of last contact. All patients provided written informed consent meeting all federal, state, and institutional guidelines.

Biomarker Analyses

We used total RNA that had been purified from primary tumor blocks received from patients in C9741 to measure the risk of recurrence score combined with tumor size and proliferation (ROR-PT), with a predefined cut point (≤50, >50), and to determine intrinsic subtype using the research version of the Prosigna hybridization assay (Nanostring Technologies, Seattle, WA).[8] We obtained permission from the National Cancer Institute (protocol CSC-0154) to receive a 300-ng aliquot (or otherwise at least 200 ng) of stored RNA from ER+ breast cancers.

The 31-gene SET2,3 index was measured from total RNA using the QuantiGene Plex (QGP) platform (Thermo Fisher Scientific, Waltham, MA) following the manufacturer's protocol, exempted under institutional review board protocol LAB04-0093 as not human subjects research.[11,12,15] Briefly, the QGP assay involves hybridization of target RNA to the oligonucleotide probes that coat specific beads, followed by signal amplification with secondary oligonucleotides and labeling with streptavidin phycoerythrin

(SAPE). The Luminex 200 instrument (Luminex, Austin, TX) counts the SAPE signals for each bead, specific to each probe. A result passed quality control for analysis if the mean count of the 10 reference genes was >3.5 (log_2 scale).[11] For prognostic interpretation, the SET2,3 index adjusts $SET_{ER/PR}$ index with a BPI derived from pathologic tumor size, number of involved lymph nodes, and molecular subtype on the basis of expression of four genes (*ESR1*, *PGR*, *ERBB2*, and *AURKA*), wherein higher BPI represents less aggressive disease.[12,17] High SET2,3 scores (≥2.10) are associated with endocrine sensitivity and more favorable prognosis, and low SET2,3 scores (<2.10) with lower endocrine sensitivity and less favorable outcomes.[17]

Statistical Analysis

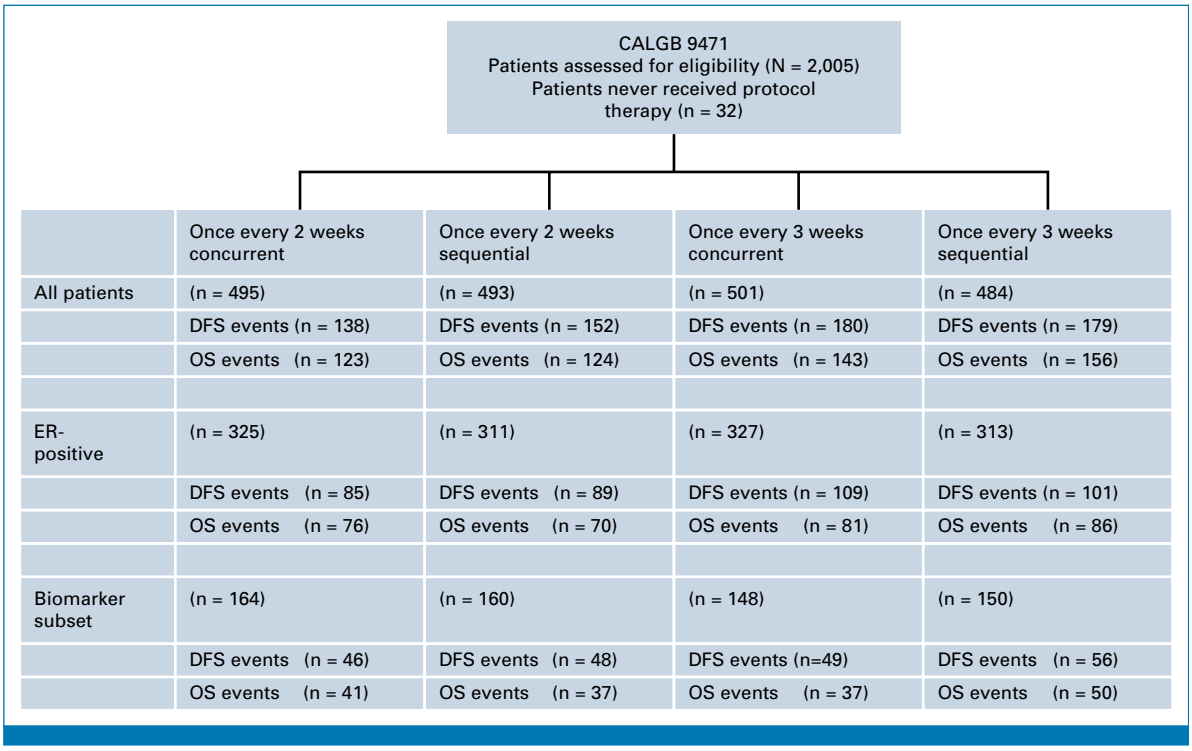
Kaplan-Meier estimators were used to generate DFS and OS curves; these were compared with a log-rank test. The a priori level of significance was a two-sided .05. SET2,3 index was evaluated as a continuous index (per unit) and categorically using the predefined cut points.[12,17] To determine whether the efficacy of chemotherapy dosing schedules depends on SET2,3 index, a Cox model was used that included SET2,3 index (continuous variable), chemotherapy intensity (dose-dense *v* conventional), and the interaction term with a prespecified level of significance for the interaction term $P < .1$. A .1 level of significance was used instead of .05 to gain additional power for detecting an interaction term given the original trial was not powered/designed to determine a test for interaction. We also sought to evaluate whether high SET2,3 was associated with favorable DFS, and to compare the SET2,3 index with the ROR-PT score, and the intrinsic subtype prognostic performance.[8] Correlation was estimated with a Pearson correlation coefficient and corresponding 95% CI. Multivariable Cox regression models that had treatment arm as a covariable were used to compare each measure of prognostic performance as a continuous value and as a categorical value using the predefined cut points.

RESULTS

Long-Term Survival Analysis

There were 649 DFS events and 546 OS events after 12 years of median follow-up of the 1,973 patients in C9741 (Fig 1). Overall, dose-dense chemotherapy reduced the relative risk of DFS event by 23% (hazard ratio [HR], 0.77 [95% CI, 0.66 to 0.90]; $P = .0009$; Fig 2A) and improved OS outcomes by 20% (HR, 0.80 [95% CI, 0.67 to 0.95]; $P = .0088$; Fig 2B), translating into absolute benefits of 6.1% for DFS and 4.7% for OS at 10 years of follow-up. The superiority of dose-dense effect remained statistically significant when adjusted for clinicopathologic factors (Data Supplement, Tables S1 and S2, online only). Treatment sequence (concurrent *v* sequential) did not influence DFS or OS (Data Supplement, Fig S1). Safety results were consistent with the initial report with no signs of increased acute leukemia during follow-up (Data Supplement, Table S3).

The benefits of dose-dense therapy were seen for both ER+ and ER− subsets, with no significant interaction between



	Once every 2 weeks concurrent	Once every 2 weeks sequential	Once every 3 weeks concurrent	Once every 3 weeks sequential
All patients	(n = 495)	(n = 493)	(n = 501)	(n = 484)
	DFS events (n = 138)	DFS events (n = 152)	DFS events (n = 180)	DFS events (n = 179)
	OS events (n = 123)	OS events (n = 124)	OS events (n = 143)	OS events (n = 156)
ER-positive	(n = 325)	(n = 311)	(n = 327)	(n = 313)
	DFS events (n = 85)	DFS events (n = 89)	DFS events (n = 109)	DFS events (n = 101)
	OS events (n = 76)	OS events (n = 70)	OS events (n = 81)	OS events (n = 86)
Biomarker subset	(n = 164)	(n = 160)	(n = 148)	(n = 150)
	DFS events (n = 46)	DFS events (n = 48)	DFS events (n=49)	DFS events (n = 56)
	OS events (n = 41)	OS events (n = 37)	OS events (n = 37)	OS events (n = 50)

FIG 1. CONSORT diagram. DFS, disease-free survival; ER, estrogen–receptor; OS, overall survival.

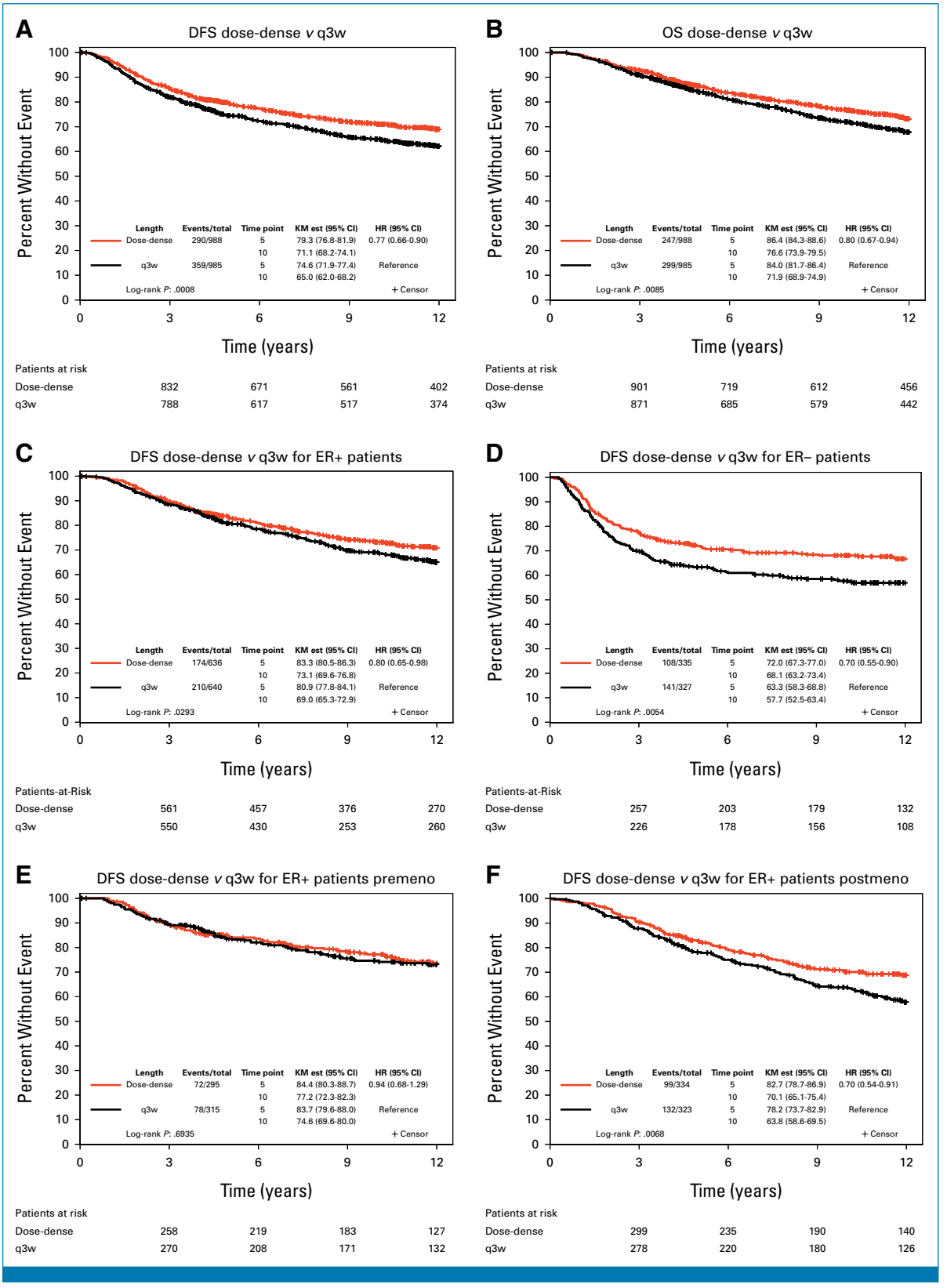


FIG 2. KM analyses of (A) dose-dense once every 2 weeks versus conventional once every 3 weeks chemotherapy regimens by DFS and (B) OS in all patients in the C9741 trial, DFS in the subsets with (C) ER+ disease and (D) ER– disease, and DFS in the subsets with (E) ER+ disease for premenopausal and (F) postmenopausal women. DFS, disease-free survival; ER+, estrogen receptor–positive; ER–, estrogen receptor–negative; HR, hazard ratio; KM, Kaplan-Meier; OS, overall survival; q3w, dose once every 3 weeks.

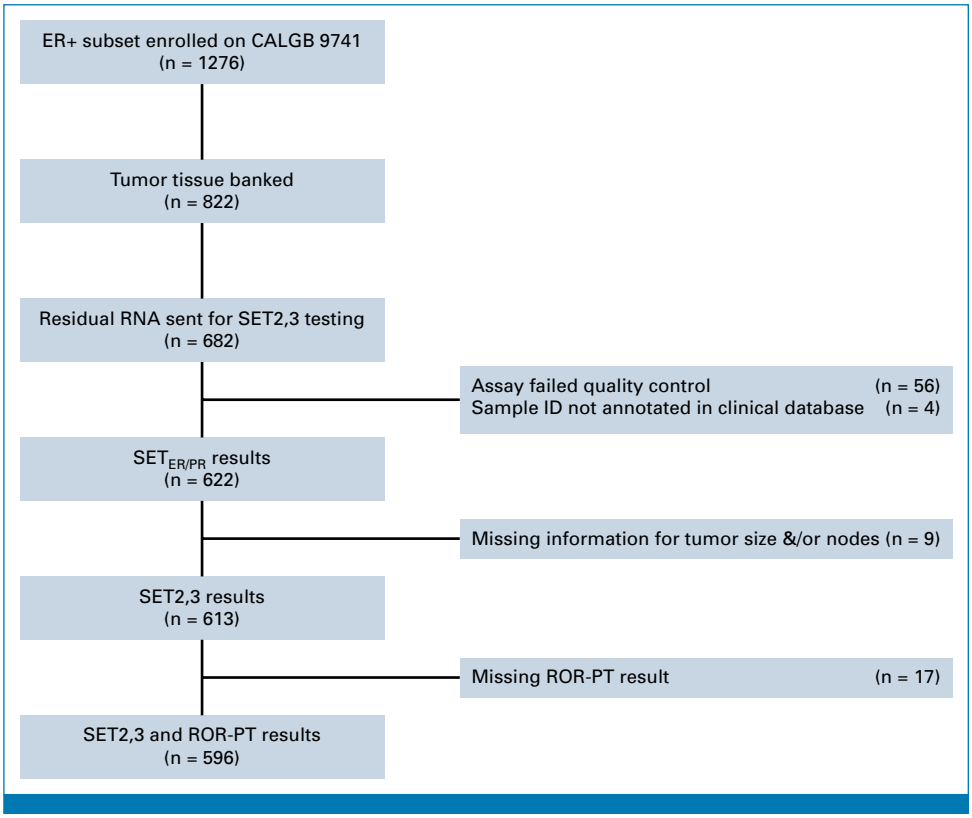


FIG 3. REMARK diagram for biospecimens and biomarker testing of the population with ER+ cancer. ER+, estrogen receptor–positive; ID, identity; PR, progesterone receptor; ROR-PT, risk of recurrence score combined with tumor size and proliferation; SET$_{ER/PR}$, sensitivity to endocrine therapy index of transcriptional activity related to estrogen and progesterone receptors.

treatment (dose-dense *v* conventional) and ER status for both DFS and OS. Dose-dense chemotherapy reduced the relative hazard of DFS event by 19.9% (95% CI, 2.1 to 34.5) for ER+ and 29.8% (95% CI, 9.8 to 45.4) for ER− disease (Figs 2C and 2D), and the relative hazard of death by 15.4% (95% CI, −5.7 to 32.2) for ER+ and 28.5% (95% CI, 6.6 to 45.2) for ER− disease.

There was no significant interaction between treatment (dose-dense *v* conventional) and menopausal status for DFS or OS in patients with ER+ breast cancer. The absolute benefit favoring dose-dense therapy was more pronounced in the postmenopausal setting, with an estimated 10-year DFS of 70.1% for dose-dense compared with 63.8% for conventional therapy (HR, 0.70 [95% CI, 0.54 to 0.91]; $P = .007$). In the premenopausal setting, the 10-year DFS estimates were 77.2% for dose-dense and 74.6% for conventional therapy (HR, 0.94 [95% CI, 0.68 to 1.29]; Figs 2E and 2F).

Risk Estimation by Genomic Risk Subsets

C9741 included 1,276 patients with ER+ breast cancer, including 822 with primary tumor sample and 682 with residual RNA available. After testing, 622 patients had evaluable genomic test results for SET$_{ER/PR}$ index, and of these, 613 patients had evaluable SET2,3 index results of the primary tumor (Fig 3). This biomarker subset had similar clinicopathologic characteristics to the overall ER+ population in C9741, except for larger tumor size (>2 cm, 60.9% *v* 50.9%) and higher rates of mastectomy (67.0% *v* 57.3%; Table 1). These differences are expected because tissue banking in this clinical trial was optional.

Using the predefined cut point (SET2,3 ≥2.10 is high, SET2,3 <2.10 is low), 40% (244/613) of tumors were classified as high SET2,3 and 60% as low SET2,3. High SET2,3 was associated with superior survival outcomes than low SET2,3. The estimate of 10-year DFS was 77.7% for high SET2,3 and 58.2% for low SET2,3 (HR, 0.38 [95% CI, 0.27 to 0.53]; $P < .0001$; Data Supplement, Fig S2A). The estimate of 10-year OS was 86.9% for high SET2,3 and 65.9% for low SET2,3 (HR, 0.37 [95% CI, 0.26 to 0.54]; $P < .0001$; Data Supplement, Fig S2B). SET2,3 index was prognostic as a continuous variable (DFS HR, 0.47 [95% CI, 0.39 to 0.58]; and OS HR, 0.38 [95% CI, 0.26 to 0.54]) in Cox models adjusted for study treatment.

PAM50 intrinsic subtypes and ROR-PT results were available for 596 of the 613 tumors with SET2,3 results. SET2,3 and ROR-PT scores had modest (negative) correlation (Pearson correlation coefficient −0.31; 95% CI, −0.38 to −0.23). Using

TABLE 1. Comparison of Baseline Characteristics of Patients With Estrogen Receptor–Positive Breast Cancer Included in This Analysis and Those Not Included

	In Analysis, No. (%)			
Characteristic	Yes (n = 622)	No (n = 654)	Total (N = 1,276)	*P*[a]
Number of positive nodes				.13851
1-3	355 (57.1)	398 (60.9)	753 (59.0)	
4-9	199 (32.0)	177 (27.1)	376 (29.5)	
≥10	68 (10.9)	77 (11.8)	145 (11.4)	
SLND only	0	2 (0.3)	2 (0.2)	
Age				.37471
≤50	287 (46.1)	318 (48.6)	605 (47.4)	
>50	335 (53.9)	336 (51.4)	671 (52.6)	
Menopausal status				.06291
Pre	281 (45.5)	329 (50.7)	610 (48.1)	
Post	337 (54.5)	320 (49.3)	657 (51.9)	
Missing	4	5	9	
Tumor size				.00041
≤2 cm	240 (39.1)	315 (49.1)	555 (44.2)	
>2 cm	374 (60.9)	327 (50.9)	701 (55.8)	
Missing	8	12	20	
Surgery				.00081
Lumpectomy	200 (32.2)	266 (40.7)	466 (36.5)	
Mastectomy	417 (67.0)	375 (57.3)	792 (62.1)	
Other	5 (0.8)	13 (2.0)	18 (1.4)	
Treatment arm				.39691
I: Sequential, every 3 weeks	150 (24.1)	163 (24.9)	313 (24.5)	
II: Sequential, every 2 weeks	160 (25.7)	151 (23.1)	311 (24.4)	
III: Concurrent, every 3 weeks	148 (23.8)	179 (27.4)	327 (25.6)	
IV: Concurrent, every 2 weeks	164 (26.4)	161 (24.6)	325 (25.5)	

Abbreviation: SLND, sentinel lymph node dissection.
[a]Chi-square *P* value.

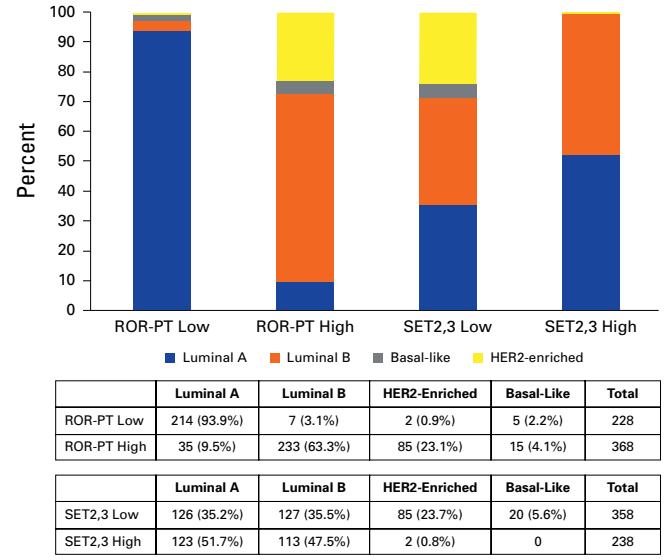
intrinsic subtypes, high SET2,3 index was observed in 49.4% (123/249) of luminal A, 47.1% (113/240) of luminal B, 2.3% (2/87) of human epidermal growth factor receptor 2 (HER2)–enriched, and none of 20 basal-like cancers (Fig 4A).

In a multivariable model in ER+ disease for DFS adjusted for study treatment, ROR-PT high versus low was associated with worse outcomes (HR, 1.40 [95% CI, 1.051 to 1.86]; $P = .023$), but when SET2,3 was added into the model the DFS estimates were significant for SET2,3 (HR, 0.46 [95% CI, 0.34 to 0.63]; $P < .0001$) and ROR-PT score lost significance (HR, 1.22 [95% CI, 0.91 to 1.64]). A similar effect was observed for OS, with SET2,3 index indicating prognosis for OS (HR, 0.36 [95% CI, 0.25 to 0.53]; $P < .0001$) but not ROR-PT (HR, 1.26 [95% CI, 0.91 to 1.75]). Kaplan-Meier plots for DFS (Fig 4B) and OS (Fig 4C) demonstrate that ROR-PT status did not contribute meaningfully to the prognostic discrimination from SET2,3 status. Similar observations were seen with models using ROR-PT score and SET2,3 index as continuous variables.

Prediction of Benefit From Dose-Dense Chemotherapy by Genomic Risk Subsets

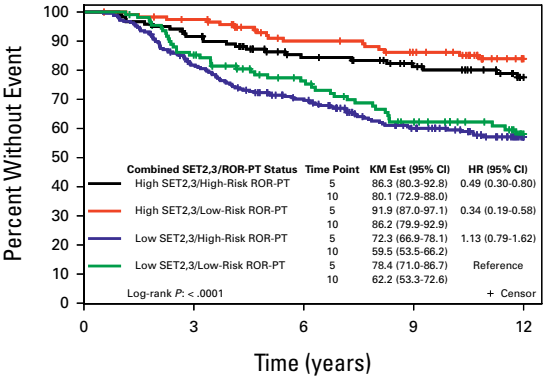
Reduction in the relative hazard for a DFS event from dose-dense chemotherapy was greater for patients with low SET2,3 (HR, 0.77 [95% CI, 0.56 to 1.04]) than for patients with high SET2,3 (HR, 0.86 [95% CI, 0.51 to 1.44]). The estimated 10-year DFS rates for dose-dense versus conventional chemotherapy were 62% versus 53.9% for patients with low SET2,3, and 78.7% versus 76.5% for patients with high SET2,3. Similar observations were seen for OS. In multivariable models including SET2,3 as continuous variable, dose-dense versus conventional regimen, and the SET2,3 by dose-dense interaction, an interaction between SET2,3 index and benefit from dose-dense chemotherapy was seen for DFS ($P_{interaction} = .0998$) and was stronger for OS ($P_{interaction} = .027$).

Low SET2,3 was more prevalent in postmenopausal women compared with premenopausal (64% *v* 56%, respectively; $P = .046$). The interaction between SET2,3 index and dose-

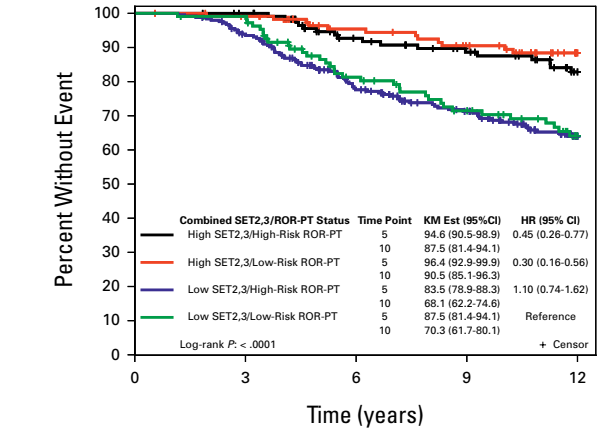


	Luminal A	Luminal B	HER2-Enriched	Basal-Like	Total
ROR-PT Low	214 (93.9%)	7 (3.1%)	2 (0.9%)	5 (2.2%)	228
ROR-PT High	35 (9.5%)	233 (63.3%)	85 (23.1%)	15 (4.1%)	368

	Luminal A	Luminal B	HER2-Enriched	Basal-Like	Total
SET2,3 Low	126 (35.2%)	127 (35.5%)	85 (23.7%)	20 (5.6%)	358
SET2,3 High	123 (51.7%)	113 (47.5%)	2 (0.8%)	0	238



Patients at risk				
High SET2,3/High-Risk ROR-PT	108	86	74	56
High SET2,3/Low-Risk ROR-PT	112	94	86	62
Low SET2,3/High-Risk ROR-PT	199	158	117	77
Low SET2,3/Low-Risk ROR-PT	91	72	55	36



Patients at risk				
High SET2,3/High Risk ROR-PT	117	94	83	65
High SET2,3/Low Risk ROR-PT	114	98	89	70
Low SET2,3/High Risk ROR-PT	228	172	140	93
Low SET2,3/Low Risk ROR-PT	106	77	64	46

FIG 4. Relationships between (A) SET2,3 index, ROR-PT score, and intrinsic subtypes as percentages of each intrinsic subtype within subsets defined by SET2,3 and ROR-PT categories for a total of 596 study participants, (B) KM analyses of the prognostic combination of SET2,3 index (high/low) and ROR-PT score (high/low) by disease-free survival, and (C) overall survival. HER2, human epidermal growth factor receptor 2; HR, hazard ratio; KM, Kaplan-Meier; ROR-PT, risk of recurrence score combined with tumor size and proliferation; SET, index of sensitivity to endocrine therapy.

dense benefit on OS was retained after adjusting for menopausal status ($P_{interaction}$ = .099 for OS). The ROR-PT score did not predict benefit from dose-dense therapy ($P_{interaction}$ = .37 for both DFS and OS). Finally, there was no differential benefit for DFS or OS by SET2,3 index or ROR-PT score by sequence (sequential *v* concurrent) of treatment.

Components of SET2,3 Index

Given the predictive interaction between SET2,3 index and survival benefit from dose-dense therapy, we sought to evaluate the predictive ability of its components ($SET_{ER/PR}$ index and BPI). Only $SET_{ER/PR}$ index had significant interaction with treatment arm on OS ($P_{interaction}$ = .030). BPI did not have a significant interaction with treatment ($P_{interaction}$ = .45). Figure 5A illustrates the relationship between the relative hazard of death between treatment arms (dose-dense *v* conventional dosing) according to the $SET_{ER/PR}$ index in the primary tumor. On the basis of this, a cut point for optimal prediction was identified as $SET_{ER/PR}$ index <0.75. Kaplan-Meier plots (Figs 5B and 5C) demonstrate improved OS from dose-dense chemotherapy for 41.3% (257/622) of patients

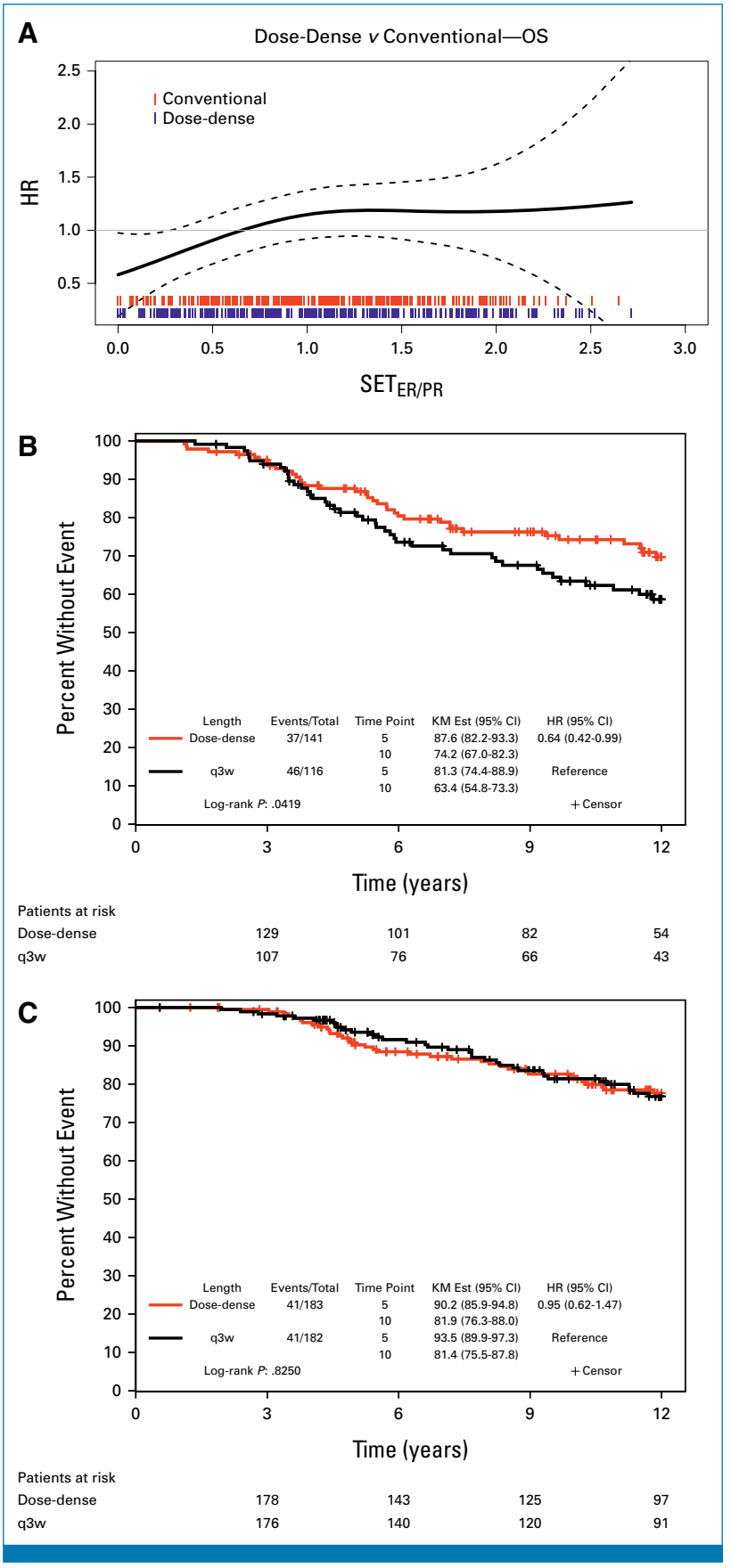


FIG 5. Exploratory analysis of $SET_{ER/PR}$ index to predict OS benefit from dose-dense chemotherapy as the hazard function (HR for (continued on following page)

FIG 5. (Continued). dose-dense *v* conventional) according to the $SET_{ER/PR}$ index value for a total of 622 patients (A); and KM analyses of OS of patients treated with dose-dense once every 2 weeks versus conventional once every 3 weeks chemotherapy regimens when the cancer has $SET_{ER,PR}$ index value <0.75 (B); or $SET_{ER,PR}$ index value of 0.75 or greater (C). ER, estrogen receptor; HR, hazard ratio; KM, Kaplan-Meier; OS, overall survival; PR, progesterone receptor; q3w, dose once every 3 weeks; ROR-PT, risk of recurrence score combined with tumor size and proliferation; $SET_{ER/PR}$, sensitivity to endocrine therapy index of transcriptional activity related to estrogen and progesterone receptors.

whose cancer had $SET_{ER/PR}$ index <0.75, but no difference for the patients with $SET_{ER/PR}$ index ≥0.75. Importantly, the predictive interaction between $SET_{ER/PR}$ index and chemotherapy treatment arm was retained after adjusting for HER2 status, defined either by intrinsic subtype ($P_{interaction}$ = .099 for DFS) or by *ERBB2* gene expression level from the SET2,3 assay ($P_{interaction}$ = .038).

DISCUSSION

The current analysis of the C9741 trial at 12 years of median follow-up confirmed the long-term benefit of dose-dense chemotherapy versus conventional regimen for patients with node-positive breast cancer, with relative risk reductions of 23% for DFS and 20% for OS regardless of ER and menopausal status. Among patients with ER+ node-positive disease, the SET2,3 index added predictive and prognostic information related to outcomes and benefit of dose-dense chemotherapy.

C9741 was designed on the basis of the principle that cytotoxic agents kill a fixed fraction of cancer cells per dose with rapid recovery of the cancer population between doses due to low-volume Gompertzian growth kinetics.[18] The current results expand on potential biological processes related to dose-dense benefit. Low level of endocrine transcriptional activity, determined by $SET_{ER/PR}$ index, identified a subgroup of patients with significant survival advantage when treated with dose-dense chemotherapy. Although the observations between $SET_{ER/PR}$ index and dose-dense benefit are aligned with our hypothesis related to cumulative cell kill, it is not unreasonable to speculate that dose-dense chemotherapy might also have a favorable effect through additional unknown factors. For example, the emergence of a treatment benefit after 5 years of median follow-up and a persistent effect throughout 12 years might implicate an off-target effect of dose-dense chemotherapy in the tumor microenvironment and is possibly relevant to immunotherapy.

To our knowledge, the $SET_{ER/PR}$ index is the first genomic assay to predict survival benefit from a dose-dense taxane-based chemotherapy regimen in ER+ disease.[7,8,19-22] We observed that proliferation-driven signatures for ROR-PT and PAM50 intrinsic subtype classification did not predict benefit from dose-dense chemotherapy, nor did the BPI component of SET2,3 index, which includes tumor size, nodal status, and molecular subtype. Importantly, $SET_{ER/PR}$ index of endocrine-related transcriptional activity predicted survival outcomes with dose-dense regimen for both pre- and postmenopausal women, even though we would expect there to be intratumoral heterogeneity in these poorly differentiated node-positive cancers.

C9741 was conducted before standard HER2 testing. Fortunately, HER2 status can be reliably imputed from *ERBB2* gene expression level or the HER2-enriched intrinsic subtype,[23,24] and HER2 status did not influence the significant interactions between $SET_{ER/PR}$ index and survival benefit from dose-dense chemotherapy.

SET2,3 index estimated risk of recurrence independently from ROR-PT score that is the basis of the Prosigna assay. This is consistent with previous studies, where SET2,3 index added independent prognostic information to contemporary prognostic tests, including the 70-gene MammaPrint (MP) and 21-gene Recurrence Score.[13,17] Indeed, SET2,3 incorporates prognostic variables of tumor size, nodal involvement, and RNA-defined molecular subtypes, but the additional core component $SET_{ER/PR}$ index adds information related to the effect of endocrine transcriptional activity.

We acknowledge the inherent limitations of a retrospective analysis of a biomarker, so further validation is warranted before using $SET_{ER/PR}$ index to facilitate chemotherapy treatment decisions. Interestingly, low SET2,3 did not predict DFS benefit from the addition of conventionally dosed anthracycline-based chemotherapy to endocrine therapy in the S8814 trial.[17] This might indicate lack of prediction of anthracycline benefit. Additionally, the $SET_{ER/PR}$ index includes the gene *MAPT* that encodes tau protein, a known inhibitor of the binding site for paclitaxel within beta tubulin structures, which has been associated with lower pathologic response rate after neoadjuvant chemotherapy.[10,25,26] Hence, it is also conceivable that $SET_{ER/PR}$ index might have predictive ability that is specific to dose density of paclitaxel.

In summary, the C9741 trial confirmed the superiority of dose-dense chemotherapy over conventionally dosed treatments across ER and menopausal subsets. The biomarker analyses presented here provide further insights that position these long-term results into a clinically relevant context. Our findings also suggest that use of a dose-dense adjuvant chemotherapy regimen should probably not be solely on the basis of measures of tumor cell proliferation or

molecular subtype. We found that ER+ cancers with low endocrine activity seem to benefit from dose-dense chemotherapy in contrast to those with high activity and independently of menopausal status.

Our findings, many years after C9741 established dose-dense anthracycline-paclitaxel chemotherapy as a standard adjuvant chemotherapy regimen, yield an unexpected long-term result in the ER+ subset, challenge the long-held paradigm that more intense chemotherapy regimens should be selected based solely on tumor burden and proliferation-driven metrics, challenge more recent inferences that postmenopausal women would not benefit, and provide new insight into the influence of endocrine activity within breast cancer cells on the rationale for dose intensity of adjuvant chemotherapy.

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DISCLAIMER

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A data sharing statement provided by the authors is available with this article at DOI https://doi.org/10.1200/JCO-24-01875. Following NCI requirements, all data will be submitted to NCI/NCTN Data Archive after publication. Researchers may also send a request to the corresponding author, Otto Metzger Filho (Otto_Metzger@DFCI.HARVARD.EDU).

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Adjuvant Dose-Dense Chemotherapy in Hormone Receptor–Positive Breast Cancer

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