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Ovarian Reserve as a Measure of Adjuvant Chemotherapy Benefit in Hormone Receptor Positive (HR+), HER2-negative, Node-positive Breast Cancer in SWOG S1007 (RxPONDER)

Kevin Kalinsky[1], **William E. Barlow**[2], **Harsh B. Pathak**[3], **Rajni V. Puri**[3], **Amrita Mitra**[3], **Trisha Home**[3], **Julie Gralow**[4], **Kathy S. Albain**[5], **Daniel F. Hayes**[6], **Suzette Delaloge**[7], **Nancy U. Lin**[8], **Edith A. Perez**[9], **Lori J. Goldstein**[10], **Stephen K.L. Chia**[11], **Priya Rastogi**[12], **Anne F. Schott**[6], **Etienne Brain**[13], **Jean-Yves Pierga**[14], **Jean-Marc Ferrero**[15], **Steven Shak**[16], **Debasish Tripathy**[17], **Gabriel N. Hortobagyi**[17], **Funda Meric-Bernstam**[17], **Priyanka Sharma**[3], **Lajos Pusztai**[18], **Alastair Thompson**[19], **Andrew K. Godwin**[3]

[1]Emory University, Atlanta, GA

[2]SWOG Statistics and Data Management Center, Seattle, WA

[3]University of Kansas Medical Center, Kansas City, KS

[4]American Society of Clinical Oncology, Alexandria, VA

[5]Loyola University Medical Center, Maywood, IL

[6]University of Michigan, Ann Arbor, MI

[7]Gustave Roussy, Villejuif, France

[8]Dana-Farber Cancer Institute, Boston, MA

[9]Mayo Clinic Jacksonville, Jacksonville, FL

[10]Fox Chase Cancer Center, Philadelphia, PA

[11]BCCA-Vancouver Cancer Centre, Vancouver, BC, CAN

[12]University of Pittsburgh School of Medicine, Pittsburgh, PA

[13]Institut Curie-Saint Cloud, Saint Cloud, France

[14]Institut Curie-Paris, Paris, France

[15]Centre Antoine Lacassagne, Université Côte-d'Azur

Corresponding Authors: Dr. Kevin Kalinsky, Department of Hematology and Medical Oncology, Winship Cancer Institute at Emory University, 1365 Clifton Road, NE, Atlanta Georgia 30322, United States of Ameria, kkalins@emory.edu; Dr. Andrew K. Godwin, Department of Pathology & Laboratory Medicine, University of Kansas Medical Center, 3901, Rainbow Blvd, Kansas City, KS 66160, United States of America, agodwin@kumc.edu.

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[16]Exact Sciences, Redwood City, CA

[17]The University of Texas MD Anderson Cancer Center, Houston, TX

[18]Yale University, New Haven, CT

[19]Baylor College of Medicine, Houston, TX

Abstract

Background: The phase 3 RxPONDER trial compared endocrine therapy (ET) alone or with chemotherapy (CET) in patients with hormone receptor-positive/HER2-negative breast cancer with recurrence score (RS) ≤25. CET benefit for the primary outcome, invasive disease-free survival (IDFS), was limited to premenopausal women who largely did not receive ovarian function suppression. In this study, we assessed hormones associated with ovarian reserve to further refine prediction of CET benefit.

Patients and Methods: Pretreatment serum estradiol, progesterone, follicular stimulating hormone (FSH), luteinizing hormone (LH), anti-Müllerian hormone (AMH), and inhibin B (INHB) were assessed in a blinded fashion from 1,556 participants aged<55. All markers used a predefined cut point. Associations with markers and IDFS and distant relapse-free survival (DRFS) were evaluated for prediction of CET benefit adjusting for RS. Cox regression analyses of assigned treatment and its interaction with potential markers were adjusted for multiplicity.

Results: Baseline estradiol, progesterone, LH, and FSH were not predictive for CET benefit. AMH≥10 pg/mL showed significant interaction with chemotherapy benefit for IDFS (p_{adj}=0.0034). In 64% of women with AMH ≥10 pg/mL (cutoff for normal ovarian reserve), iDFS was superior with CET compared to ET alone (HR=0.46; 95% 0.33-0.65; p_{adj}=0.00012), whereas CET was not beneficial in the 36% with low ovarian reserve (AMH <10 pg/mL) (HR=1.27; 95% 0.81-1.99; p_{adj}=0.47). DRFS showed a similar pattern of results for AMH. Ultrasensitive INHB measured in the pg/mL range was also predictive of CET benefit.

Conclusion: Among premenopausal patients aged<55, participants with baseline AMH <10 pg/mL did not benefit from CET. AMH was a significantly better indicator of CET benefit than menopause status, age, or other hormones. Measures of ovarian reserve may refine selection of patients for CET.

Keywords

RxPONDER; SWOG S1007; Oncotype; Recurrence Score; Anti-Mullerian Hormone; Ovarian Reserve

Background

Postmenopausal women with hormone receptor positive (HR-positive), human epidermal growth factor receptor 2 negative (HER2-negative) breast cancer with 1-3 positive nodes and 21-gene Recurrence Score (RS)≤25 can safely forgo chemotherapy and receive endocrine therapy alone (ET) after breast surgery without compromising invasive-disease free survival (IDFS), based on results from the S1007 trial (RxPONDER)[1]. In RxPONDER, premenopausal women aged≤50 years benefit from chemotherapy followed by endocrine

therapy (CET), independently of RS. In contrast, premenopausal women aged≥50 had a lower magnitude of chemotherapy benefit. It is also possible that some women were misclassified by menopausal status due to menopausal status being based on historical definitions. Taken together, these results suggest that CET benefit observed in premenopausal women with HR-positive/HER2-negative breast cancer might be due to the ovarian suppressive effect as opposed to direct anti-tumor activity[2, 3]. In RxPONDER, usage of ovarian function suppression was low in premenopausal participants, with only 6% and 19% in the CET and ET arms, respectively[1]. Therefore, there is an unmet need to further refine who benefits from CET to avoid overtreatment in younger women.

Anti-Müllerian Hormone (AMH) is a well-established measure of ovarian reserve[4,5,6]. AMH is produced by granulosa cells of the preantral and small antral ovarian follicle[7]. Circulating AMH levels associate with the number of growing ovarian follicles and, as a result, reproductive potential and the timing of natural menopause [8, 9]. Higher AMH levels indicate greater ovarian reserve, whereas lower AMH levels suggest diminished ovarian reserve[4]. Because AMH levels remain relatively stable throughout the menstrual cycle, AMH is considered a reliable biomarker for assessing ovarian reserve and infertility[4, 5, 10].

Inhibin B (INHB) regulates follicle-stimulating hormone (FSH) secretion and reflects ovarian reserve and ovarian function[11]. This glycoprotein hormone is secreted from granulosa cells of developing antral follicles and reflects ovarian follicular quantity[12]. Similar to AMH, serum INHB levels decline with age as both the quantity and quality of ovarian follicles decrease[13].

Relevant to this study, both AMH and INHB provide endocrine profiles of key stages during the menopause transition[14]. AMH levels decrease as early as five years before the final menstrual period (FMP), representing a critical biological transition point[15]. Low or undetectable INHB levels measured using conventional assays are also observed during this period and may become undetectable two to three years prior to menopause[15]. Tests for AMH and INHB used for fertility testing are typically performed using assay with lower limits of detection in the ng/mL range. In contrast, for this study we used ultrasensitive clinically validated assays with detection limits in the low pg/mL ranges.

We evaluated the role of serum hormone levels in women aged<55 in determining CET benefit in RxPONDER. We hypothesized that premenopausal patients with HR-positive/HER2-negative early-stage breast cancer and limited ovarian reserve, as determined by ultrasensitive AMH and/or INHB measurements, would not benefit from chemotherapy. In this setting, these biomarkers may be more informative than menopausal status, age, or other hormone levels, including estradiol, progesterone, LH, and FSH.

PATIENTS AND METHODS

Population

In RxPONDER, 5,083 women with HR-positive/HER2-negative BC, 1-3 positive nodes, and RS≤ 25 were randomly assigned to CET or ET. The design and results have been previously published[1]. Participants were classified as premenopausal if the last menstrual

period (LMP) was <6 months since study entry or postmenopausal if a) previous bilateral oophorectomy or b) LMP >12 months and no previous hysterectomy. If these definitions did not apply, participants were categorized as premenopausal if aged<50 or postmenopausal if aged≥50. The National Cancer Institute (NCI) approved an amendment to evaluate baseline hormone levels to further discriminate chemotherapy benefit if aged<55[1]. Age 55 was selected because approximately 85% of women have undergone menopause by this age[16].

Cohorts

Three separate cohorts of participants aged<55 were assayed consecutively (Figure 1). NCI-sponsored participants were classified as premenopausal (Cohort 1) or postmenopausal (Cohort 2). Results for Cohort 1 provided evidence that supported assaying the remaining cohorts. Cohort 3 included participants in S1007 whose serum was collected from UNICANCER comprehensive cancer centers after the first two cohorts Results were analyzed for all participants, then separately by premenopausal and postmenopausal status at registration. Blood collection was only at time of trial registration, and not timed to any phase of the menstrual cycle

Assay measurement

Magnetic bead-based fluorescent immunoassays from Millipore were used to measure levels of LH, FSH, estradiol, and progesterone and colorimetric ELISAs from Ansh Labs were used to measure AMH and INHB levels on blinded serum samples via the Biomarker Discovery Laboratory (BDL) at the University of Kansas Medical Center (KUMC). The details on specific assays used in this study are described in the Supplementary Materials section. Per the manufacturer of the ultrasensitive MenoCheck picoAMH ELISA, low AMH <10 pg/mL associates with being at the last menstrual period or later (*i.e.,* biochemically postmenopausal); medium AMH between 10 pg/mL and 100 pg/mL with <5 years from the LMP; and high AMH >100 pg/mL with at least 5 years from the LMP. The lower limit of detection was 1.3 pg/mL for AMH and 0.77 pg/mL for the ultrasensitive INHB assay. Values below these were set to the lower limit for quantitative analysis. To assess how these research assays performed relative to clinical assays, we performed interlaboratory comparisons with CLIA laboratories at the KU Health System (Kansas City, KS) for LH, FSH, estradiol and progesterone, Sinochips Diagnostics (Olathe, KS) for AMH, and ARUP Laboratories (Salt Lake City, UT) for both AMH and INHB using test sample sets (Table S1). The specific assays used by the clinical laboratories for the correlation studies are described in the Supplementary Materials. Notably, ARUP Laboratories uses the same MenoCheck picoAMH assay as our laboratory, whereas Sinochips Diagnostics uses a Roche AMH assay. AMH levels showed a strong concordance between our measurements and both clinical assays (Table S1). Use of human samples was approved by the KUMC Institutional Review Board.

Statistical methods

IDFS was defined as the time from date of randomization to date of first invasive recurrence (local, regional, or distant), new invasive primary cancer (breast cancer or another type of cancer), or death from any cause. Distant relapse–free survival (DRFS) was defined as the time to distant recurrence or death from any cause. Analyses were conducted in the

intention-to-treat population of eligible participants by their assigned treatment. Statistical methods included multivariable Cox models with testing the interaction of hormone level (dichotomous or continuous) and treatment assignment adjusting for the highly significant prognostic marker continuous RS. Continuous hormone values were log10 transformed for all analyses. For statistical comparisons assessing chemotherapy benefit or its interaction with biomarkers, we used the Benjamini and Hochberg (1995) approach to control the false discovery rate and give p-values adjusted for multiplicity (shown as p_{adj}). For the analysis of postmenopausal women and exploratory analyses, only hazard ratios and 95% confidence intervals were presented due to the small number of IDFS events and since no chemotherapy benefit was expected in the postmenopausal population.

RESULTS

Serum Hormone Levels in Women Aged<55

Of the 2,125 eligible participants aged<55, 1,556 (73.2%) had baseline serum available for analysis (Figure 1). Those with serum were more likely premenopausal at trial entry (n=1,221) than those without serum but did not differ in tumor characteristics (Table S2). Of the 1,221 premenopausal participants 1,101 (90.2%) had LMP < 6 months; 116 (9.5%) were classified as premenopausal based on age < 50 without evidence otherwise; and 4 (0.3%) had contradictory evidence. Of the 335 postmenopausal participants, 259 (77.3%) were classified based on a previous bilateral oophorectomy or LMP >12 months and no previous hysterectomy; 73 (21.8%) did not have those characteristics but were 50 years or older; and 3 (0.9%) had contradictory evidence. Post-randomization treatments in the first year are shown in Table S3 and confirm few patients received ovarian function suppression (OFS). Median follow-up time was 8.0 years with 232 IDFS and 138 DRFS events.

RS was not correlated with age or any log10 hormone level (Table S4). Age was significantly correlated with all hormone levels ($p<0.001$), with the highest correlation with AMH ($r=-0.73$). All hormone levels were significantly correlated ($P<0.001$) with each other in pairwise comparisons, including AMH with FSH ($r=-0.75$) and AMH with INHB ($r=0.73$). Hormone levels in premenopausal participants aged<55 are shown for AMH (Figure 2) and for estradiol, progesterone, FSH, LH, and INHB (Figures S1-S5).

For all women aged<55 with available serum, there was an overall chemotherapy benefit in IDFS (HR=0.65; $p_{adj}=0.0066$, 95% CI 0.50-0.85). However, some subsets did not benefit. Women aged<50 had chemotherapy benefit (HR=0.47; $p_{adj}=0.0004$, 95% CI 0.33-0.67) while women aged 50-54 did not (HR=1.02; $p_{adj}=0.95$, 95% CI 0.68-1.54), with a significant interaction of age<50 and treatment ($p_{adj}=0.020$). Premenopausal women aged<55 had chemotherapy benefit (HR=0.55; $p_{adj}=0.0009$, 95% CI 0.41-0.75) while postmenopausal women did not (HR=1.18; $p_{adj}=0.72$, 95% CI 0.67-2.07), with a marginal interaction of menopausal status and treatment ($p_{adj}=0.056$).

To further refine CET benefit, we evaluated individual hormone markers as either continuous or categorical variables interacting with assigned treatment, using a multivariable Cox model in all women aged<55 (Table S5). Estradiol and progesterone were not predictive of chemotherapy benefit. LH was weakly predictive as a dichotomous marker ($p_{adj}=0.035$),

while AMH and INHB were strongly predictive using either a dichotomous cutoff (p_{adj}=0.0034 and p_{adj}=0.0036, respectively) or continuous log10 measure (p_{adj}=0.0034 and p_{adj}=0.0036, respectively). The preferred models for prediction of IDFS chemotherapy benefit was either AMH or INHB interacting with assigned treatment, adjusting for continuous RS.

Ovarian Reserve in Women Aged<55

Using pre-established cut points, ultrasensitive AMH assay levels of <10 pg/mL, consistent with postmenopausal range, were identified in 558 participants (35.9%) (Figure 2), with 445 (79.8%) of those with AMH < 10 at the limit of detection. Testing the interaction of this cutoff (10 pg/mL) with treatment arm showed AMH was a significant predictor of chemotherapy benefit (p_{adj}=0.035), adjusting for RS. For the 998 patients with AMH≥10 pg/mL, there was an IDFS benefit for those assigned to CET versus ET (HR=0.46; 95% CI: 0.33-0.65, p_{adj}=0.0001), with an absolute 5-year IDFS chemotherapy benefit of 8.5% (Figure 3A). For those with low AMH, there was no chemotherapy benefit (HR=1.27; 95% CI: 0.81-1.99, p_{adj}=0.47) (Figure 3B). Similar results were observed for DRFS. For AMH ≥10 pg/mL, there was significant chemotherapy benefit (HR=0.44; 95% CI: 0.28-0.69, p_{adj}=0.0001) (Figure 3C), with an absolute 5-year DRFS benefit of 3.8%, and no chemotherapy benefit for low AMH (HR=1.51: 95% CI: 0.85-2.70, p_{adj}=0.27) (Figure 3D) (interaction p_{adj}=0.0049).

In women aged<55, 745 (56.3%) had low INHB levels, defined as being within postmenopausal range using the predetermined cutoff of 12 pg/mL measured with an ultrasensitive assay. Only participants with INHB≥12 pg/mL derived benefit from chemotherapy (HR=0.39; 95% CI 0.26-0.59; p_{adj}=0.0001) (Figure 4A), adjusting for RS, whereas those with low INHB did not (HR=1.00; 95% CI: 0.70-1.43, p_{adj}=0.997) (Figure 4B) (interaction p_{adj}=0.0036). A similar pattern was observed with DRFS, with chemotherapy benefit in patients if INHB≥12 pg/mL (Figure 4C) but not in those with <12 pg/mL (Figure 4D), although the interaction did not reach statistical significant (p_{adj}=0.058). These findings support the potential clinical relevance of ultrasensitive INHB measurement as an indicator of ovarian reserve and chemotherapy benefit, similar to AMH.

AMH and INHB showed equivalent predictive ability for chemotherapy benefit when considered as continuous variables. However, a joint model with both did not improve the overall model fit due to their high collinearity. Participants with AMH≥10 pg/mL but low INHB<12 pg/mL, demonstrated numeric benefit from chemotherapy after adjusting for RS (HR=0.62; 95% CI 0.35-1.09, p_{adj}=0.17) although not statistically significant (Figure S6).

In a sensitivity analysis, we also fit a joint model of AMH with age (<50 vs. 50-54) and menopausal status as additional predictive variables. All interaction terms with treatment became nonsignificant due to high overlap of these variables (interaction with AMH p_{adj}=0.08; interaction with age <50 (p_{adj}=0.56); and interaction with menopausal status p_{adj}=0.97), indicating little predictive benefit from a joint model. Nonetheless, we present some additional stratified analyses of AMH by menopausal status at enrollment and age group below.

Serum AMH and Chemotherapy Benefit in Premenopausal Women Aged<55

Among the 1,221 classified premenopausal women aged<55 (Figure 1), there were 183 IDFS and 108 DRFS events. In this population, 252 (20.6%), 290 (23.8%), and 679 participants (55.6%) had low, medium, and high AMH, respectively (Figure S7). Notably, low AMH was identified in 80/887 (9.0%) participants aged<50 versus 172/334 (51.5%) aged 50-54 (Figure S8).

The IDFS hazard ratio for CET versus ET for women classified as premenopausal was 0.55 (95% CI 0.41-0.75), indicating chemotherapy benefit (p_{adj}=0.0009), adjusting for RS. AMH was a significant predictor of chemotherapy benefit if AMH≥ 10 pg/mL (HR=0.46; 95% CI 0.33-0.65; p_{adj}=0.0012) (Figure 5A), while those with low AMH did not (HR=1.21; 95% CI: 0.60-2.43), p_{adj}=0.73) benefit (Figure 5B) (interaction p_{adj}=0.035), adjusting for RS in both groups. Similar findings were observed with DRFS. For women with AMH≥10 pg/mL there was chemotherapy benefit for DRFS (HR=0.46; 95% CI 0.29-0.72, p_{adj}=0.0036) (Figure 5C) but not if AMH <10 pg/mL (HR=1.51; 95% CI 0.62-3.65, p_{adj}=0.53) (Figure 5D) (interaction p_{adj}=0.051), adjusting for RS in both groups. When adjusting for age, treatment arm, and RS, AMH remained predictive for DRFS chemotherapy benefit (Table S6).

Serum AMH and Chemotherapy Benefit in Postmenopausal Women Aged<55

Among the 335 classified postmenopausal women aged<55, there were 49 IDFS and 30 DRFS events. Of these women, 29/335 (8.7%) had levels ≥10 pg/mL. For those aged 50-54 (n=283), 268 (94.7%) had low AMH versus 38/52 (73.1%) women aged<50 (Figure S9). Despite a smaller number of events, the HRs for chemotherapy benefit were similar to premenopausal women although underpowered. The IDFS HR for CET versus ET was 0.62 (95% CI 0.05-7.33) if AMH≥10 pg/mL and 1.29 (95% CI 0.71-2.32) if AMH<10 pg/mL, respectively, adjusting for RS (Figure S10).

Exploratory Analyses of Chemotherapy Benefit by Age Group and AMH

Despite the strong predictive benefit of AMH overall, there is an exception in the 117 women aged <40 of whom 116 (99.2%) have high AMH. In this youngest age group with 25 IDFS events, the IDFS HR for CET versus ET was 0.92 (95% CI 0.41-2.05), adjusting for RS. In women aged 40-44, the IDFS HR for CET versus ET was 0.36 (95% CI 0.20-0.66) with most having elevated AMH 296/311 (95.2%). For women 45-49, the overall treatment effect is HR=0.43 (95% CI 0.25-0.75). In those with low (71.3%) and elevated (80%) AMH, the HR was 0.57 (95% CI 0.10-3.10) and 0.42 (95% CI 0.23-0.75), respectively. For women aged 50-54, the overall treatment effect was HR=1.03 (95% CI 0.68-1.53). In those with low (20%) and elevated (28.7%) AMH, the HR was 1.37 (95% CI 0.84-2.22) 0.51 (95% CI 0.23-1.11), respectively.

DISCUSSION

In RxPONDER, premenopausal women with 1-3 positive nodes and RS≤25 have improved IDFS and DRFS with CET compared to ET. Our hypothesis was that ovarian reserve would be more predictive than age or menopausal status to define chemotherapy benefit. Notably, we found that women with low pre-treatment serum AMH were unlikely to benefit from

chemotherapy even if less than age 50. Similarly, women aged 50+ could potentially benefit provided AMH≥10 pg/mL. Overall, we found ovarian reserve was a significantly better indicator of adjuvant chemotherapy benefit than self-reported menopause status, age, or other hormone levels (*i.e.,* estradiol, progesterone, LH, FSH).

In RxPONDER, blood collection was not timed to any phase of the menstrual cycle. For estradiol, progesterone, FSH, and LH, this variability may limit the utility of these hormones when measured at random time points for assessing baseline ovarian reserve or predicting chemotherapy benefit. However, it has been shown that although all 6 markers vary to different extents within a menstrual cycle, changes in AMH levels are minimally influenced by the menstrual cycle phase[17]and any fluctuations are not sufficient enough to have meaningful effect on clinical interpretation of ovarian reserve. Although INHB levels vary throughout a menstrual cycle, the threshold used in the current study for ovarian reserve is far lower than typical levels measured in premenopausal women during a menstrual cycle. These findings underscore the rationale for prioritizing AMH and INHB over the traditional serum hormones for monitoring ovarian reserve/menopause status. In accordance, LH, FSH, estradiol and progesterone did not show a statistically significant association with chemotherapy-related IDFS benefit in our study whereas AMH and INHB did.

AMH does not vary significantly during menstrual cycles[17] and is only marginally affected by exogenous hormones, such as hormonal contraceptives[18]. In a small prospective trial, pre-chemotherapy AMH levels, but not age, FSH, or LH, independently predicted loss of ovarian function at 2 years post-chemotherapy[19]. In a systematic review, AMH did not return to pre-treatment levels after chemotherapy, particularly alkylating agents, and high pre-treatment AMH and lower age were predictors of having some, though not complete, recovery of AMH levels[20]. Compared to older premenopausal patients for whom AMH might be more reliable in predicting menstrual function[21], younger women might continue to have menstrual periods despite limited ovarian reserve and low AMH[20].

In the current study, low AMH was defined as <10 pg/mL, based on the manufacturer-established threshold for the ultrasensitive MenoCheck picoAMH ELISA. Although AMH levels were highly correlated among our laboratory at KUMC, ARUP Laboratories using same Ansh Labs ELISA, and Sinochips Diagnostics using the clinical Roche AMH assay (Table S1), it is important to note that the Roche assay has a limit of detection of ~30 pg/mL. Therefore, while several commercial assays for AMH are available, to our knowledge, they do not reliably quantify the very low AMH concentrations (<10 pg/mL) identified as clinically informative in this study. Furthermore, the cut points used in our analyses were prespecified by the assay manufacturer (*i.e.,* 10 pg/mL for AMH and 12 pg/mL for INHB). These prespecified cut points may not be optimal, especially for INHB, and additional exploration studies could further refine the optimal AMH and INHB threshold thresholds for identification of premenopausal women who may safely avoid CET.

Although others have reported that cessation of menstrual periods after chemotherapy is associated with DFS[2, 3, 22, 23], our data suggest that menstrual status is not an accurate measure of ovarian reserve or therapeutic benefit. The finding that patients with limited ovarian reserve do not benefit from CET in RxPONDER is consistent with the hypothesis

that a large part of CET benefit in premenopausal women is mediated by chemotherapy-induced menopause. However, there have also been unique genomic and transcriptomic alterations in tumors from younger premenopausal women that might lead to worse outcomes than older premenopausal women, suggesting that the biology of tumors in younger women might be different[24-26]. RxPONDER was not designed to formally evaluate whether chemotherapy can be replaced by OFS, especially given the low rate of OFS use in premenopausal women in this trial, including in the ET arm (<20%)[1]. Rather, the results demonstrate that there are premenopausal patients with tumors that develop in the milieu of limited ovarian reserve that do not benefit from chemotherapy and might elect to opt-out of CET without compromising their outcome. Whether OFS can be used in lieu of chemotherapy is being prospectively addressed in premenopausal women in the ongoing NRG-BR009 randomized trial (OFSET), as all participants receive OFS plus ET and are randomized to chemotherapy or not based on the RS[27], as well as OPTIMA-YOUNG, utilizing they PAM50 assay (clinicaltrials.gov: NCT0710663). While there have been previous attempts to address this issue in trials prior to the utilization of genomic assays, they were stopped early due to low accrual[28, 29]. Also, our results demonstrate that postmenopausal women with elevated AMH levels might benefit and therefore opt-in to CET; though, this was a small population of postmenopausal participants (9.1%).

INHB is another measure of ovarian reserve; however, pre-treatment INHB is less well-studied in breast cancer and does not consistently associate with menses resumption compared to AMH[30-32]. Various assays with different cutoffs have been used in prior studies. While pre-treatment AMH and INHB predict chemotherapy benefit in this analysis, AMH offered the strongest chemotherapy prediction (Table S4). While a joint model with both hormone levels did not improve the overall model for chemotherapy prediction, it might be that combining AMH and INHB with optimized assays and cutoffs could further refine who benefits most from CET.

Menopausal status was a stratification variable at randomization. However, each participant's menopausal status was not independently verified by the study team, and there could be variability between investigators in assessing this status. While subsequent endocrine and ovarian suppression treatment might further discriminate clarity of menopausal status, this study was intent-to-treat and used only information collected before or at randomization to determine whether a participant was pre- or post-menopausal. In an exploratory analysis, we noted that chemotherapy benefit was less evident if age <40 despite all but one patient having elevated AMH. In TAILORx, younger women had less chemotherapy benefit likely due to resumption of periods[33]. Given the small numbers of these cohorts in our analysis, however, these data should be interpreted as hypothesis-generating.

Our study has a few limitations. In RxPONDER, serial serum samples were not prospectively collected and thus, longitudinal assessment of ovarian reserve and differences in hormone levels after CET and ET cannot be measured. In addition, gynecologic co-morbidities, such as polycystic ovary syndrome which can associate with elevated AMH levels[34], were not reliably collected and might have influenced measures of ovarian reserve. Furthermore, this study involves multiple comparisons, which might lead to

over-interpretation of positive results. Thus, these findings deserve further validation in additional completed or new clinical trials addressing this hypothesis. Furthermore, while clinically validated assays for AMH and Inhibin B are widely available and routinely used in reproductive medicine, their application has largely focused on higher concentration ranges relevant to fertility assessment. Consequently, the analytical performance of these assays at the lower limits of detection, particularly in postmenopausal populations, is not well-characterized. A key goal of this study is to prompt clinical laboratories, including those using alternative assay platforms, to reassess and optimize assay performance in this lower range to enable more reliable identification of women with minimally detectable ovarian reserve.

We report that premenopausal women aged<55 with 1-3 positive axillary lymph nodes and RS ≤25 were able to safely forgo chemotherapy without compromising IDFS and DRFS if they have low pre-treatment serum AMH levels <10 pg/mL measured using an ultrasensitive ELISA assay. This subgroup represented 20.6% of premenopausal women aged<55 in RxPONDER. These finding conclude that pretreatment serum AMH levels measured using ultrasensitive assays capable of detecting concentrations <10 pg/mL may improve identification of women who can safely forego adjuvant chemotherapy when menopausal status is uncertain.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Premenopausal patients aged < 55 with low baseline AMH <10 pg/mL did not have IDFS or DRFS improvement with chemotherapy.
- Baseline estradiol, progesterone, LH, and FSH were not predictive of chemotherapy benefit.
- Ultrasensitive Inhibin B measured in pg/mL range was also predictive of chemotherapy benefit.
- Baseline measures of ovarian reserve might further inform who benefits from chemotherapy.

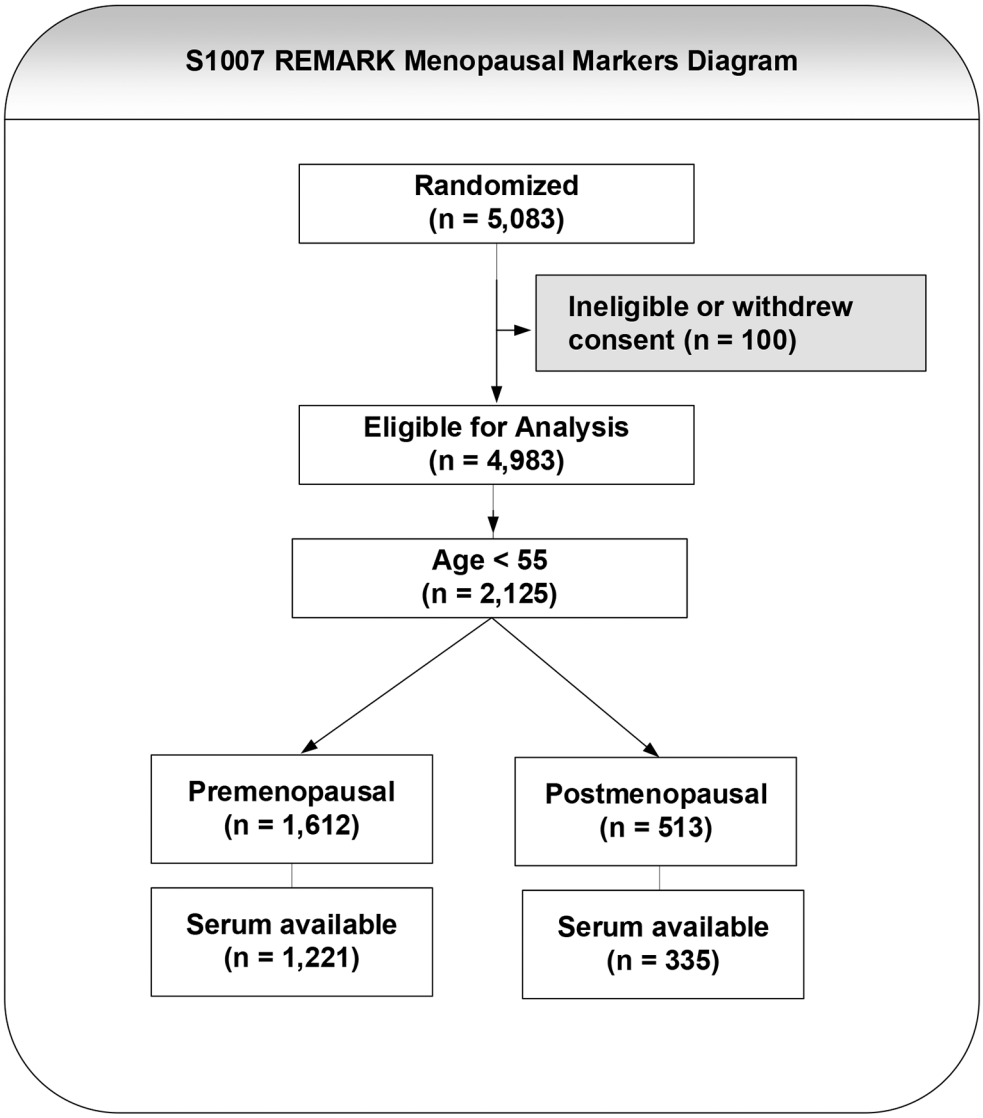

Figure 1. Consort Diagram.

Of the 5,083 participants in RxPONDER, 2,125 women age < 55 were eligible serum available and eligible for analysis.

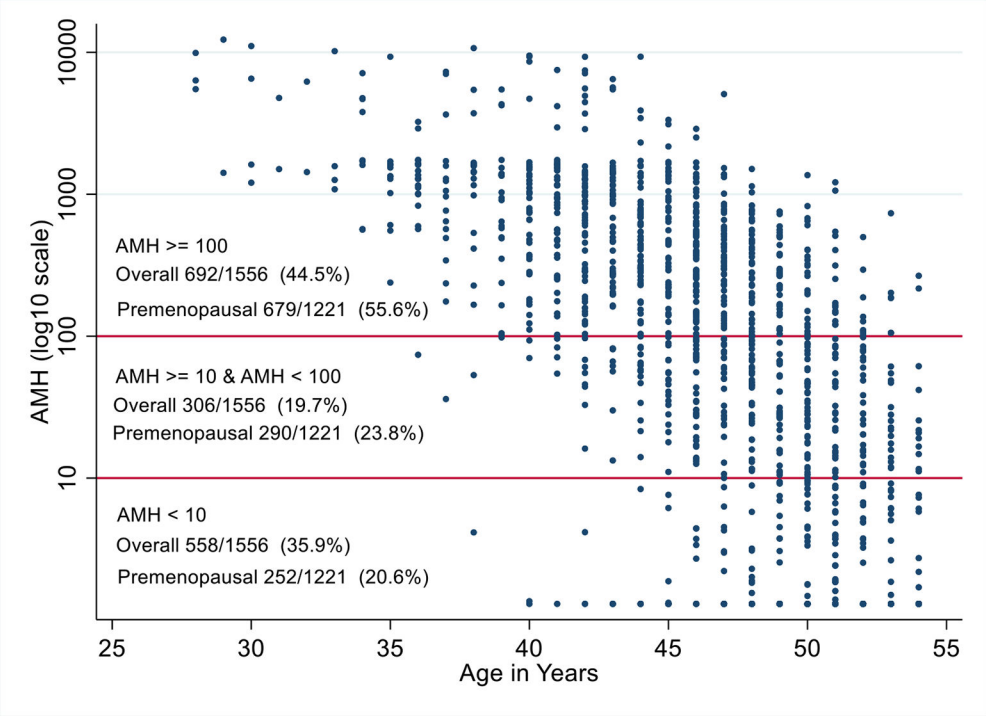

Figure 2. Baseline Serum Anti-Mullerian Hormone Levels (AMH) by Age for All Participants Age < 55.

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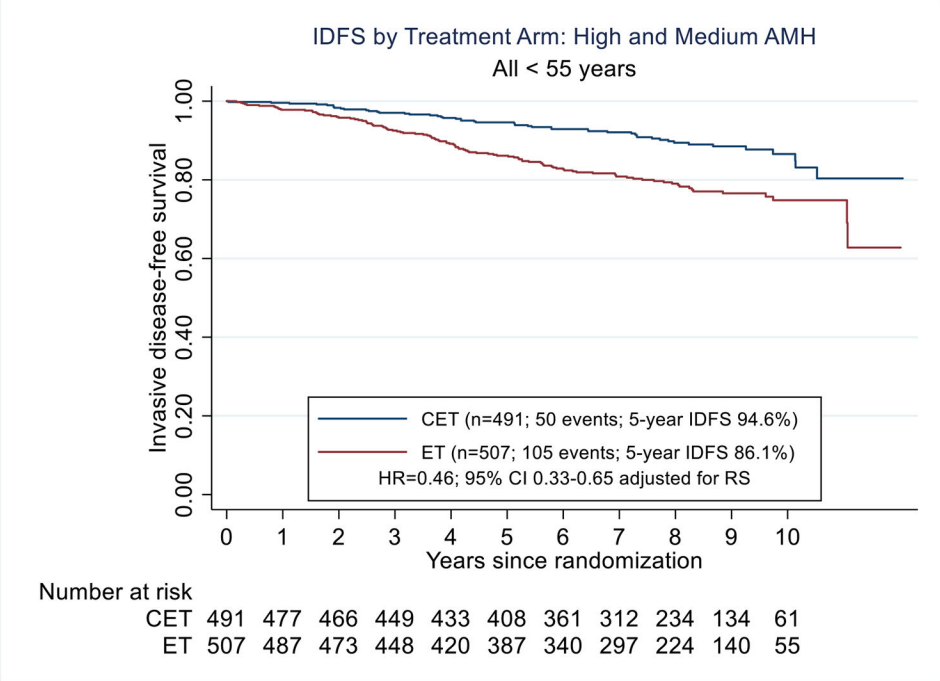

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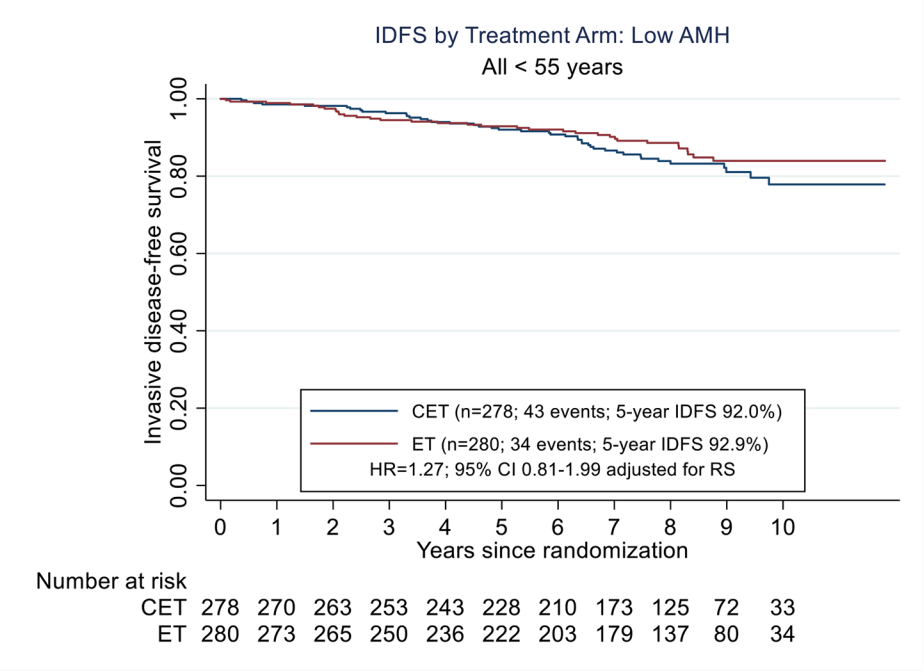

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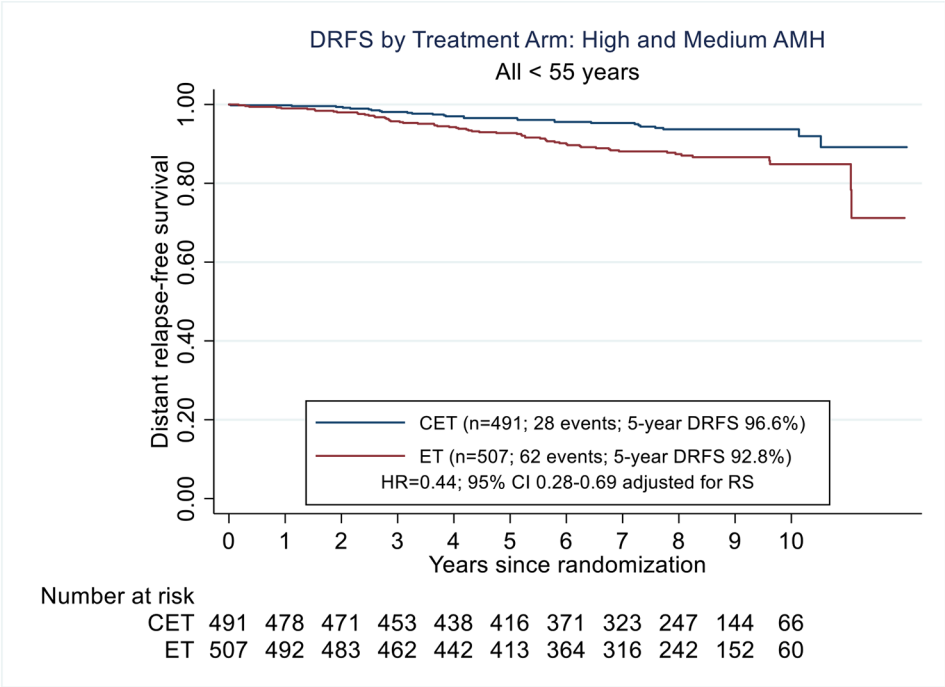

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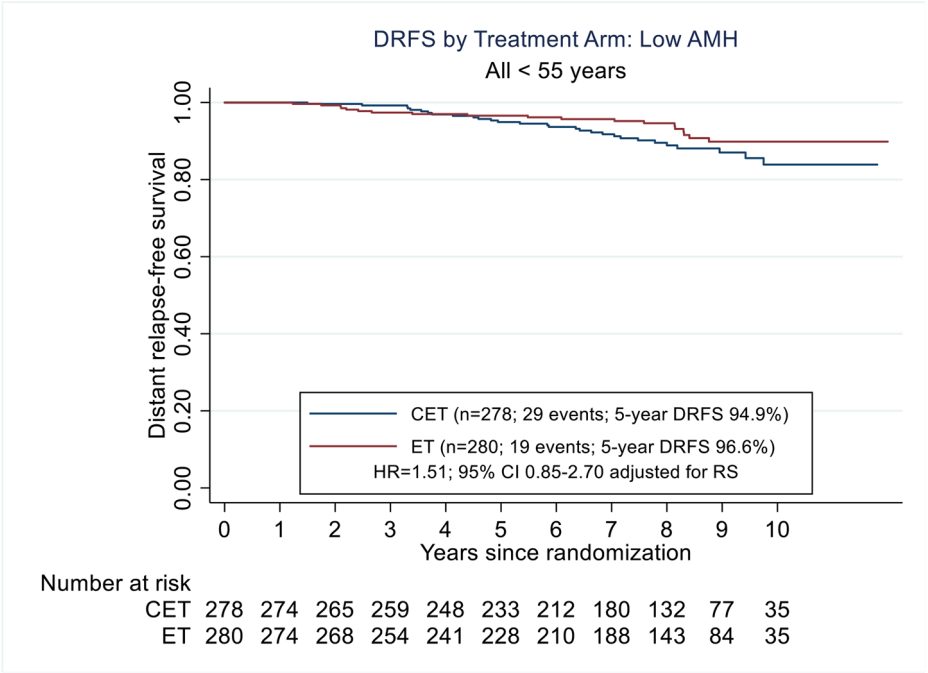
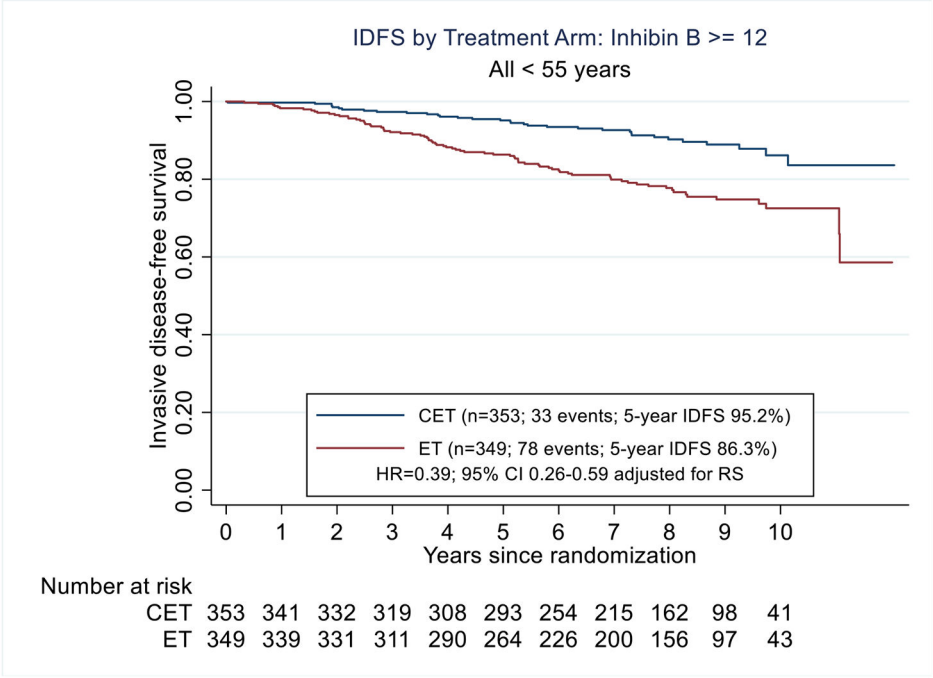


Figure 3. Invasive Disease-Free Survival (IDFS) and Distant Relapse-Free Survival (DRFS) by treatment arm among All Participants Age < 55 with a Recurrence Score of 25 or Lower and According to Serum Anti-Mullerian Hormone (AMH) Level.

A, B) IDFS by either high/medium, ≧ 10 pg/mL (A) or low, < 10 pg/mL (B) AMH levels. **C, D**) DRFS by either high/medium, ≧ 10 pg/mL (C) or low, < 10 pg/mL (D) AMH levels. All Hazard Ratios (HRs) comparing CET (chemo-endocrine therapy) and ET (endocrine therapy alone) shown in the figure were adjusted for the continuous Recurrence Score (RS). CI denotes confidence interval.

A.



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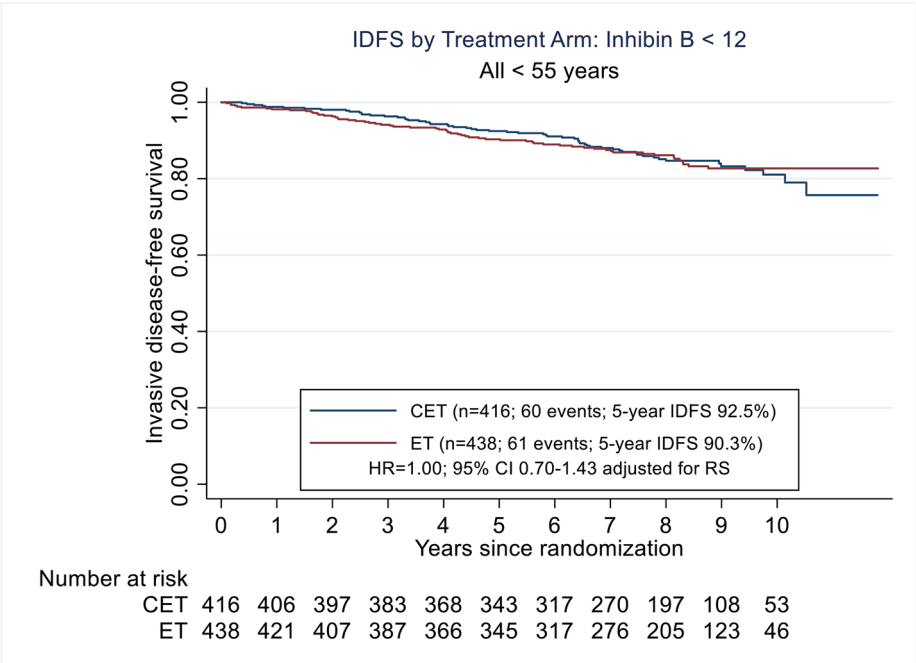

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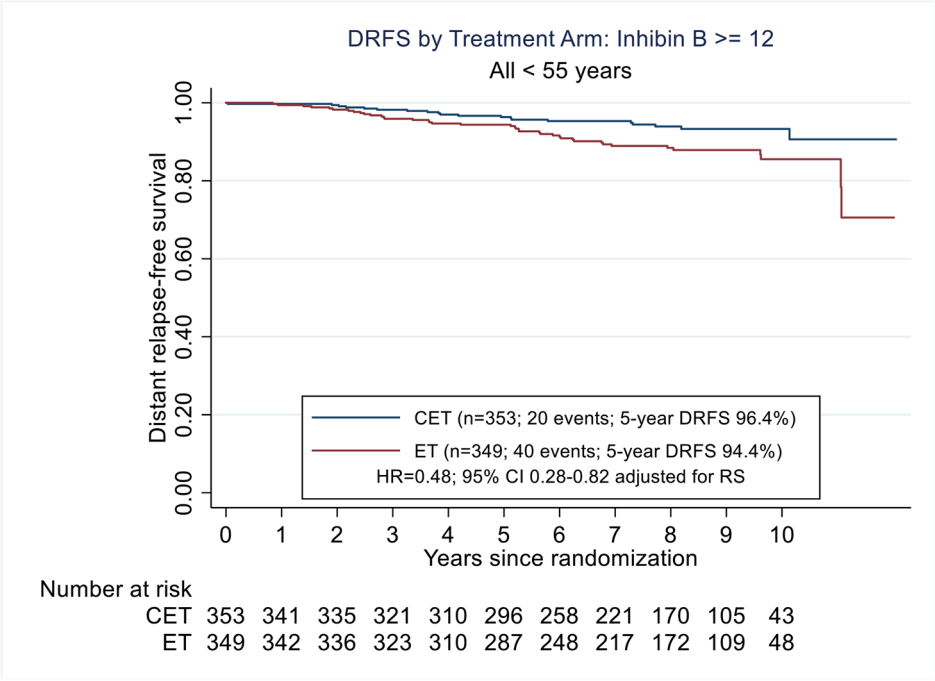

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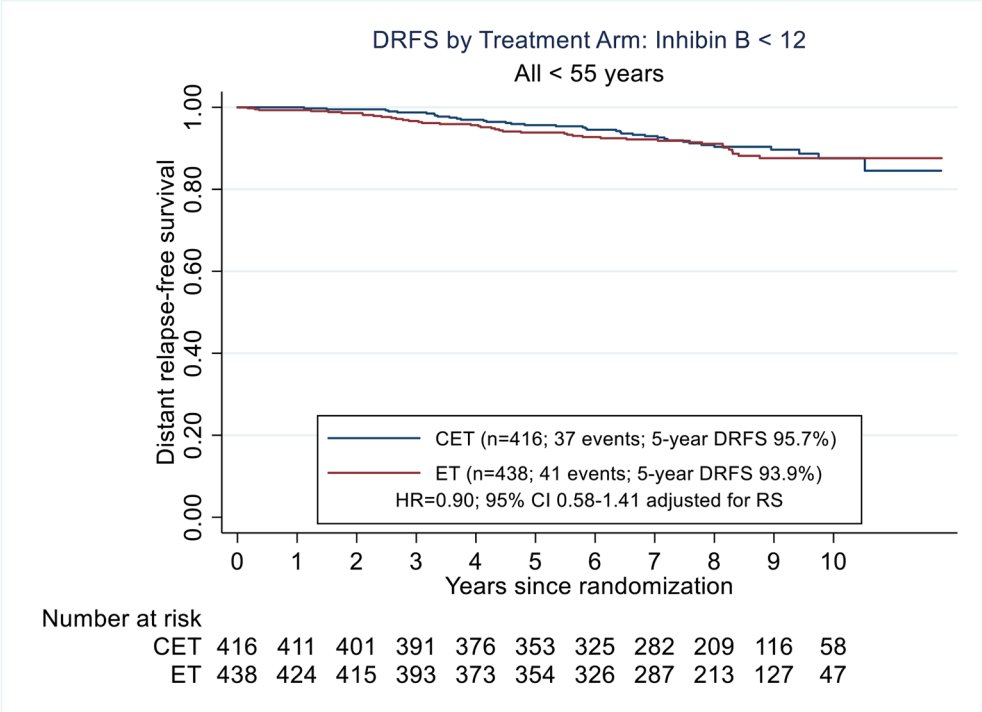
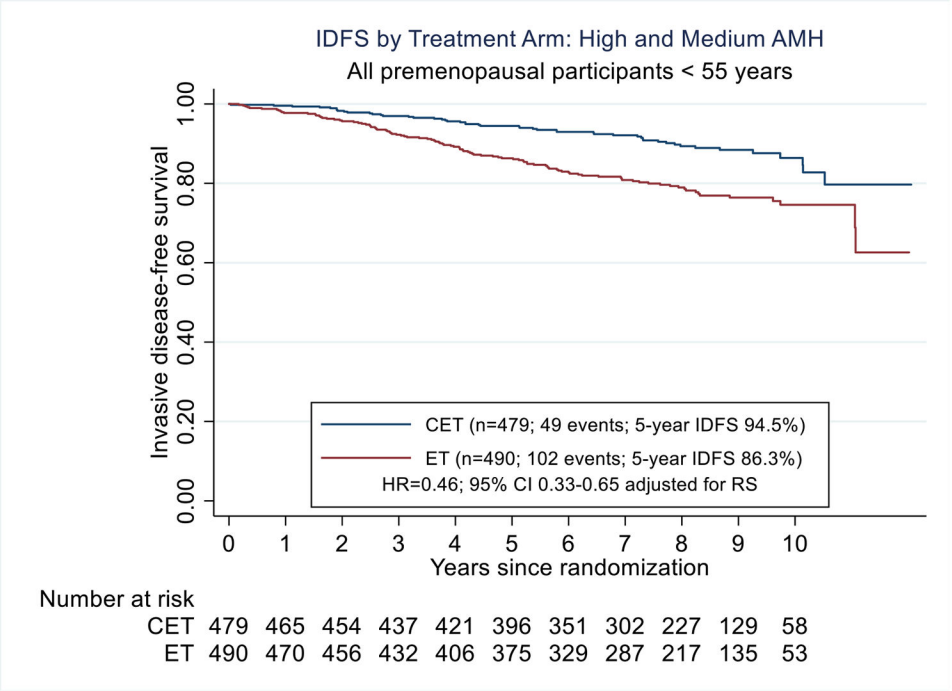


Figure 4. Invasive Disease-Free Survival (IDFS) and Distant Relapse-Free Survival (DRFS) by treatment arm among All Participants Age < 55 with a Recurrence Score of 25 or Lower and According to Inhibin B Level.

A, B) IDFS by either high, ≥ 12 pg/mL (A) or low, < 12 pg/mL (B) Inhibin B levels. **C, D**) DRFS in premenopausal women by either high, ≥ 12 pg/mL (C) or low, < 12 pg/mL (D) Inhibin B levels. All Hazard Ratios (HRs) comparing CET (chemo-endocrine therapy) and ET (endocrine therapy alone) shown in the figure were adjusted for the continuous Recurrence Score (RS). CI denotes confidence interval.

A.



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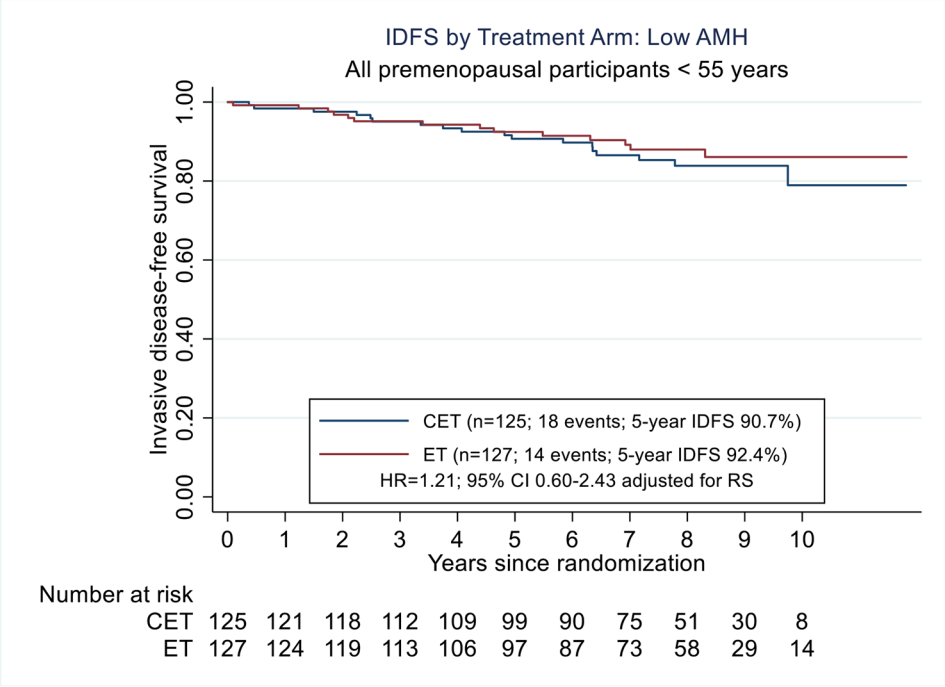

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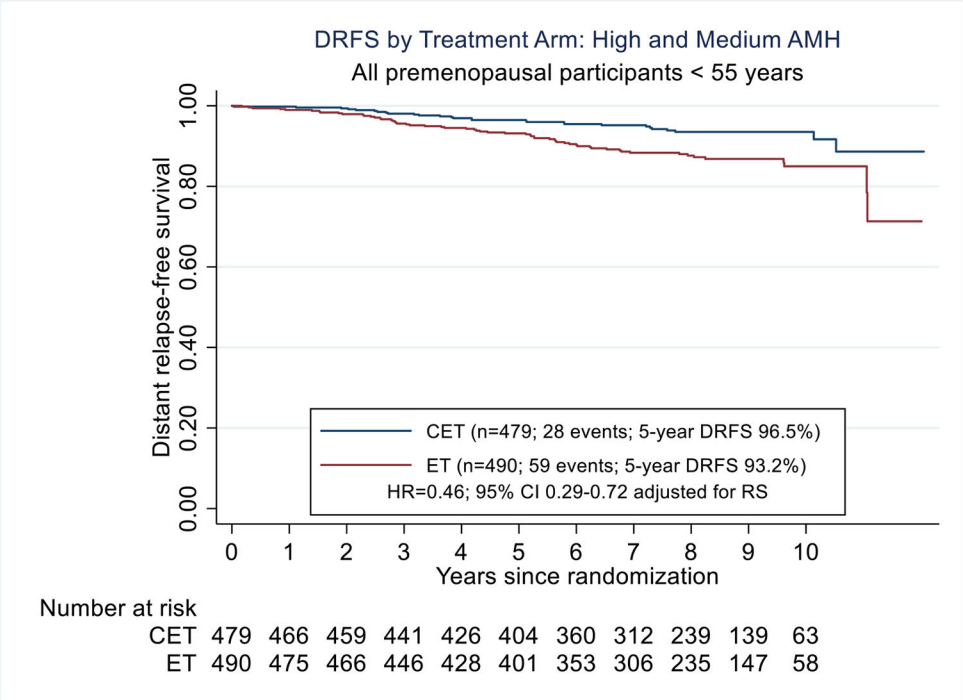

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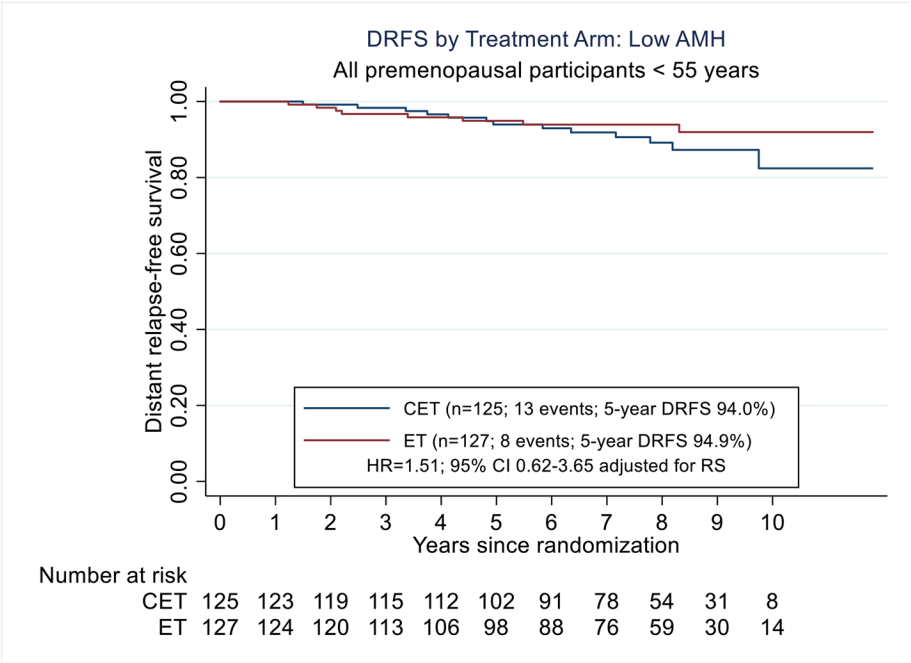

Figure 5. Invasive Disease-Free Survival (IDFS) and Distant Relapse-Free Survival (DRFS) by treatment arm among Participants Aged < 55 Classified as Premenopausal with a Recurrence Score of 25 or Lower and According to Serum Anti-Mullerian Hormone (AMH) Level.

A, B) IDFS in premenopausal women by either high/medium, ≥ 10 pg/mL (A) or low, < 10 pg/mL (B) AMH levels. **C, D**) DRFS in premenopausal women by either high/medium, ≥ 10 pg/mL (C) or low, < 10 pg/mL (D) AMH levels. All Hazard Ratios (HRs) comparing CET (chemo-endocrine therapy) and ET (endocrine therapy alone) shown in the figure were adjusted for the continuous Recurrence Score (RS). CI denotes confidence interval.