FOXC1 Expression Predicts Capecitabine Efficacy in Patients with Triple-Negative Breast Cancer from the GEICAM_CIBOMA Trial

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ABSTRACT

Purpose: In a prespecified GEICAM_CIBOMA trial (NCT00130533) correlative analysis, PAM50 non–basal-like breast cancer (non-BLBC) status distinguished patients with triple-negative breast cancer (TNBC) who are most likely to benefit from adjuvant capecitabine. The standardized forkhead box C1 (FOXC1) IHC test has demonstrated strong reliability in classifying the BLBC subtype throughout TNBC cohorts. This translational analysis aimed to evaluate the prognostic/predictive significance of BLBC classification by FOXC1 IHC in the phase III GEICAM_CIBOMA clinical trial.

Experimental Design: Tumor tissues from patients with TNBC randomized to standard (neo)adjuvant chemotherapy followed by capecitabine versus observation were analyzed using the standardized FOXC1 IHC test to assess its BLBC/non-BLBC TNBC subtyping capacity as a distant relapse-free survival clinical outcome predictor of capecitabine benefit (exploratory endpoints: disease-free survival, overall survival, and recurrence-free survival).

Results: A total of 705 (80.5%) patients from the GEICAM_CIBOMA trial were evaluable for FOXC1 expression analysis, with balanced distribution between the trial's treatments. FOXC1 proportion/intensity (VFOXC1) score–based subtyping demonstrated a strong association [AUC = 0.87; 95% confidence interval (CI), 0.84–0.91] and agreement (κ index = 0.43; $P < 0.0001$) with PAM50 molecular subtyping. VFOXC1 non-BLBC TNBC subtype was a significant independent predictor of clinical benefit with capecitabine for distant relapse-free survival (HR, 0.44; 95% CI, 0.25–0.76; $P = 0.003$). This predictive effect of VFOXC1 non-BLBC on capecitabine efficacy was further confirmed at disease-free survival (HR, 0.47; 95% CI, 0.28–0.78; $P = 0.003$), overall survival (HR, 0.48; 95% CI, 0.24–0.96; $P = 0.038$), and recurrence-free survival (HR, 0.39; 95% CI, 0.22–0.72; $P = 0.002$).

Conclusions: This ambispective GEICAM_CIBOMA translational analysis validated FOXC1-based basal-like/non–basal-like subtyping as a pragmatic alternative to PAM50 subtyping and independently predicted the benefit of adding capecitabine to standard (neo)adjuvant chemotherapy in TNBC.

Introduction

Triple-negative breast cancer (TNBC) represents approximately 10% to 20% of diagnosed breast cancer cases and is associated with a poorer prognosis and more aggressive behavior than other breast cancer subtypes. This clinical denomination encompasses a heterogeneous group of diseases differing at histopathologic, genomic, immunologic, and clinical levels (1). Currently, chemotherapy (CT)

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Translational Relevance

Prior evidence demonstrated that PAM50 non–basal-like breast cancer (non-BLBC) status identifies patients with triple-negative breast cancer (TNBC) who are most likely to benefit from adjuvant capecitabine. However, alternative attainable methods to identify non-BLBC TNBC are needed. The standardized forkhead box C1 (FOXC1) IHC test reliably identifies BLBC within TNBC cohorts. This hypothesis-testing study evaluates the standardized FOXC1 IHC capacity to predict benefit from extended adjuvant capecitabine on the GEICAM_CIBOMA trial. Following REMARK criteria, our findings align FOXC1 IHC accuracy with gold-standard PAM50 subtyping, demonstrating that FOXC1 non-BLBC subtype defines patients with TNBC who are most likely to benefit from adjuvant capecitabine compared with those with BLBC tumors. These results suggest that FOXC1 IHC assessment could enhance oncologists' ability to precisely select patients with non-BLBC TNBC for optimal treatment. Further validation in larger prospective–retrospective clinical trials is needed to achieve level 1B evidence. Nevertheless, these findings may guide clinical decisions and study designs for patients with TNBC, particularly those with residual disease after neoadjuvant therapy.

remains the cornerstone of systemic therapy for patients with early-stage TNBC, especially with anthracycline- and taxane-based regimens being the most frequently administered agents in the adjuvant and neoadjuvant settings (2). Furthermore, immunotherapy has emerged as a promising therapeutic approach for early TNBC because of its highly immunogenic nature (3), suggesting that it may become a standard component of (neo)adjuvant treatment regimens (4), although no evidence of benefit has been demonstrated to date in the adjuvant setting (5).

The incorporation of capecitabine in the adjuvant setting of TNBC has been evaluated in several clinical trials attempting to improve breast cancer outcomes (6, 7). Notably, a meta-analysis of individual patient data from 12 randomized clinical trials, assessing the benefit of capecitabine in either neoadjuvant or adjuvant setting, demonstrated a significant improvement in both disease-free survival (DFS) and overall survival (OS) when capecitabine was added to standard CT in patients with TNBC compared with those with non-TNBC (8).

The GEICAM/2003-11_CIBOMA/2004-01 phase III clinical trial (GEICAM_CIBOMA trial; NCT00130533) explored extended adjuvant capecitabine treatment after the completion of standard (neo)adjuvant CT in patients with early TNBC. Although this randomized trial failed to show a statistically significant improvement in DFS in the whole cohort by adding 6 months of adjuvant capecitabine, a preplanned subset analysis revealed a notable improvement in DFS and OS for patients with non–basal-like breast cancer (non-BLBC) TNBC tumors, which were defined by the absence of EGFR and cytokeratin 5/6 (CK5/6) staining (9). Gene expression analysis of tumor samples from the GEICAM_CIBOMA trial further identified the PAM50 non-BLBC subtype as the most significant predictor of capecitabine benefit (10). This observation was corroborated by a preplanned analysis in the ECOG-ACRIN EA1131 trial, which also identified that patients with non-BLBC had superior invasive DFS when treated with capecitabine compared with those treated with a platinum-based agent (invasive DFS, 65% with capecitabine vs. 46% with platinum; ref. 11). These results suggest that distinguishing between BLBC and non-BLBC within TNBC tumors may be essential for selecting patients who would benefit from capecitabine-based adjuvant treatment.

Given the predictive value of PAM50 non-BLBC subtype for capecitabine benefit, alternative and attainable methods to accurately define non-BLBC within TNBC are needed. Several studies have shown that Forkhead box protein C1 (FOXC1) expression, a biologically relevant transcription factor, could effectively identify BLBC within TNBC subtype (12, 13). Elevated FOXC1 expression has been associated with poor outcomes in various cancers, including breast cancer (14). Moreover, the standardized FOXC1 IHC test (VERESCA test) is a semiquantitative standardized IHC assay developed by Onconostic Technologies-3N Diagnostics (OT-3NDx), designed to detect FOXC1 protein overexpression in formalin-fixed, paraffin-embedded (FFPE) breast carcinoma tissue. This test has shown a strong capacity to identify BLBC subtype throughout TNBC cohorts, including scientific evidence of its accuracy to identify BLBC subtype across TNBC cohorts, and has been shown to be accurate when compared with PAM50-defined BLBC subtype (gold standard; ref. 15). Its prognostic potential was independently proved in a cohort of 250 patients with TNBC previously treated with the docetaxel plus carboplatin regimen (16).

In the present prospective–retrospective translational study, we aim to evaluate the ability of the standardized FOXC1 IHC assay to classify TNBC tumors as BLBC or non-BLBC and predict the benefit of extended adjuvant capecitabine treatment following the standard (neo)adjuvant CT in patients from the GEICAM_CIBOMA phase III randomized clinical trial.

Materials and Methods

Study population

GEICAM_CIBOMA (ClinicalTrials.gov identifier: NCT00130533) is a multicenter, open-label, randomized phase III clinical trial that was conducted in 80 centers across 8 countries (Spain, Brazil, Chile, Colombia, Ecuador, Mexico, Peru, and Venezuela) between October 2006 and September 2011 (9). A total of 876 patients with breast cancer were recruited; these included women, ages ≥18 and ≤70 years, with a histologically centrally confirmed invasive breast adenocarcinoma and TNBC status defined by IHC as negative for estrogen receptor (ER; <1%), progesterone receptor (PR; <1%), and HER2 following the most current guidelines established by the American Society of Clinical Oncology and the College of American Pathologists at the time of recruitment (17, 18). Eligible patients were those with ipsilateral axillary lymph node involvement classified as pN1a, pN2a, or pN3a (excluding metastatic infraclavicular lymph nodes) or those without axillary node involvement (N0) with a primary tumor size ≥1 cm. The trial received approval from the independent ethics committees or institutional review boards at each of the participating institutions.

During the GEICAM_CIBOMA trial, an IHC-defined preplanned stratum for BLBC versus non-BLBC (IHC phenotype by CK5/6 and EGFR staining) was performed centrally by the GEICAM Spanish Breast Cancer Group on surgically resected, pretreatment FFPE tumor samples from the randomized patients. BLBC/non-BLBC classification was scored as previously published (19). A subsequent prespecified correlative gene expression analysis was conducted on this TNBC trial to determine the gold-standard PAM50 intrinsic subtypes, enabling the classification of patients as PAM50 BLBC or

non-BLBC (10). Patients treated with surgery and standard (neo) adjuvant CT were randomly assigned to capecitabine versus observation. Patients assigned to adjuvant capecitabine received 8 cycles of oral capecitabine 1,000 mg/m^2 twice daily on days 1 to 14 of each 21-day cycle. Full details on the GEICAM_CIBOMA study treatment protocols have been reported (9).

Study design and endpoints

The current study follows a formal prospective–retrospective design per REMARK criteria (20) and per the guidelines for the use of archived clinical trial specimens for predictive biomarker evaluation on clinical trials (21). Upon approval by the ethics committees or institutional review boards of all participating sites, all patients signed a written informed consent to participate in the GEICAM_CIBOMA trial, allowing the use of their tumor tissue for study-related research purposes. The use of patients' specimens without the disclosure of identifiers complied with the waiver of informed consent policy criteria in accordance with the ethical guidelines of the Declaration of Helsinki. Archival FFPE tumor tissue samples were obtained at the time of surgery before adjuvant therapy from patients enrolled in the GEICAM_CIBOMA trial. An analysis plan was prespecified to explore the predictive and prognostic capacity for BLBC/non-BLBC categories obtained from the standardized FOXC1 IHC test (OT-3NDx), alone or in combination with the Ki67 index.

The primary endpoint of the GEICAM_CIBOMA trial was DFS defined as the time from random assignment to locoregional or distant recurrence, second primary malignancy, or death, whichever occurred first [definition coincident with current invasive DFS (22)]. OS was one of the secondary endpoints of the original analysis, defined as the time from the date of randomization to the date of death from any cause. Given that the DFS definition included second primary malignancies (non-breast) reported to be higher on the observation arm compared with capecitabine (3% vs. 1.3%) in GEICAM_CIBOMA trial patients and that the reduction in DFS events with capecitabine was mainly due to distant relapses among IHC non-BLBC cases, the current correlative study uses the primary endpoint of distant relapse-free survival (DRFS) to avoid a potential reporting bias (capecitabine does not seem to be associated with second primary malignancy incidence in this trial; ref. 9). DRFS is defined as the time from randomization to distant recurrence of breast cancer (documented deaths due to breast cancer without distant recurrence were also considered a distant recurrence event; local recurrence, regional recurrence, and contralateral second primary or secondary breast cancer in the ipsilateral breast are not considered distant recurrence events). DFS and OS were used as secondary endpoints in the current correlative study. Additionally, recurrence-free survival (RFS) was included as a secondary endpoint to encompass for distant, local, and regional recurrence events while excluding second primary tumors.

Standardized FOXC1 IHC test and Ki67 immunostainings

The standardized FOXC1 IHC test (FOXC1 mouse mAb B2E3 primary antibody; VERESCA test, OT-3NDx) was the semiquantitative IHC assay performed in GEICAM_CIBOMA trial tumors to determine FOXC1 protein expression following the manufacturer's instructions (15). Briefly, the IHC staining procedure was performed on a Dako Autostainer Link 48 (Agilent Technologies) platform. Tissue sections (4 μm) were obtained from the archival FFPE tumors. Heat-induced epitope retrieval was performed using diluted EnVision FLEX Target Retrieval Solution, High pH on a PT Link at 95°C for 30 minutes. Primary antibody anti-FOXC1(B2E3) 1 μg/μL, 1:50 dilution, was incubated for 20 minutes at room temperature. Specimens were incubated with the FLEX Mouse LINKER for 15 minutes, followed by 20-minute incubation with the secondary anti-mouse EnVision FLEX Detection System. Normal human salivary gland tissue served as both positive and negative controls in each staining run. Sections incubated with normal, nonimmunized rabbit immunoglobulins were used as additional negative controls.

Ki67 nuclear protein expression was assessed with the standardized IHC test using Monoclonal Mouse Anti-Human Ki-67 Antigen (Clone MIB-1, Dako Omnis-Agilent) according to the manufacturer's recommendations and following the latest international recommendations for Ki67 assessment in breast cancer (23). High proliferation in breast cancer based on Ki67 labeling by IHC was defined following the 18th St. Gallen International Breast Cancer Conference (2023) criteria based on a proliferation threshold ≥20% (24).

FOXC1 proportion/intensity score

The FOXC1 proportion/intensity (VFOXC1) score was developed to enable a semiquantitative analysis of FOXC1 expression, ensuring standardization, consistent interpretation, uniformity, and concordance to aid in the diagnostic assessment of BLBC (15, 25). Previous FOXC1 IHC scoring development and validation with the standardized FOXC1 IHC test were previously conducted by three independent, blinded pathologists. They assessed nuclear staining intensity and the percentage of positive cancer cells using predefined increments, with values stratified in 10% intervals, following established "H" and Allred scoring guidelines for ER and PR (15, 26). FOXC1 expression was determined exclusively based on nuclear IHC staining patterns. First, tumor sections were assessed for the proportion of tumor cells exhibiting positive nuclear staining for FOXC1 using a scale from 0 to 5 [0: none; 1: 0%–1%; 2: 1%–10%; 3: 11%–33%; 4: 34%–66%; and 5: 67%–100%; proportion score (PS)]. Next, sections were evaluated for staining intensity on a scale from 0 to 3 [0: none; 1: weak; 2: intermediate; and 3: strong; intensity score (IS)].

The VFOXC1 score was calculated as the sum of PS and IS, resulting in a total score ranging from 0 to 8. As previously performed, a cutoff point of VFOXC1 ≥4 was used to distinguish BLBC (high VFOXC1) from non-BLBC (low VFOXC1) subtypes (15, 27, 28).

Prior to this translational study cohort, 38 TNBC samples from the Fundación Jiménez Díaz University Hospital Biobank were assessed for FOXC1 staining using the standardized FOXC1 IHC test as quality and scoring validation. Two independent pathologists (FR and SPB), using this IHC test standardized scoring method, achieved an almost perfect agreement (κ index > 0.88) in FOXC1 evaluation (data not shown).

Statistical analysis

The prespecified approved statistical analysis plan was independently executed by the statisticians of the GEICAM headquarters, testing the prognostic and predictive capacity of VFOXC1 and additional BLBC/non-BLBC category variables. ROC curves and AUC were used to evaluate the performance of the VFOXC1 score as a continuous score to predict the BLBC/non-BLBC categories across different definitions. Interassay reliability among BLBC/non-BLBC classification methods (PAM50 subtyping, IHC phenotype, and VFOXC1) was quantified using the κ concordance index. Kaplan–Meier survival curves showing the survival distribution among

BLBC/non-BLBC category variables (PAM50 subtyping, IHC phenotype, and VFOXC1) are depicted, and the *P* value of the log-rank test was reported. Univariate and multivariate survival analyses were performed for continuous and categorized variables using Cox regression models (primary and secondary endpoints). In all the Cox regression models, calculated in the whole study cohort and in each treatment arm, the HRs were computed along with its 95% confidence interval (95% CI), and the *P* value was reported. Multivariate analysis was adjusted for age at randomization, menopausal status, histologic grade, tumor size, stage, breast surgery, region (Spain vs. Latin America), nodal status, and CT regimen. Interaction tests of heterogeneity that assess the associations of variable categories with clinical outcomes between treatment arms were used.

All statistical tests were conducted at the two-sided 0.05 level of significance using R statistical software.

Data availability

The aggregated data generated in this study are included in this article. Additional data are available from the corresponding author upon reasonable request.

Results

Of the 876 women enrolled in the GEICAM_CIBOMA trial, 744 (84.9%) had tumor tissue samples available. Of these, 705 (80.5%) were evaluable for FOXC1 protein expression using the standardized FOXC1 IHC test, defining the translational study cohort (**Fig. 1**). Specimens with suboptimal fixation, tissue autolysis, or the absence of endogenous FOXC1 staining in basal cells at normal breast ducts (used as an internal positive control) were excluded. Among the 705 evaluable cases, 357 (50.6%) patients received capecitabine, whereas 348 (49.4%) were assigned to the observation arm. There were no imbalances in clinicopathologic characteristics between the two populations (χ^2 test $P > 0.05$; **Table 1**).

The standardized FOXC1 IHC staining revealed that FOXC1 expression was distributed throughout the tumor, with primary staining located in the nuclei of tumor cells (**Fig. 2A**). Faint FOXC1 expression was also detected in the adjacent nontumoral breast epithelium, in basal cells of breast ducts, and in some stromal cells. In the study cohort, 186 (26.4%) tumors showed no evaluable FOXC1 nuclear staining (PS = 0; IS = 0). A total of 299 (42.4%) cases exhibited a high proportion of positive nuclear FOXC1 staining, including 150 (21.3%) cases with 34%–66% stained tumor cells (PS = 4) and 149 (21.1%) cases with ≥67% stained cells (PS = 5). Staining intensity was evenly distributed across the cohort, with 179 (25.4%) specimens showing weak staining (IS = 1); 184 (26.1%) specimens showing intermediate staining (IS = 2); and 156 (22.1%) specimens exhibiting strong staining patterns (IS = 3; Supplementary Table S1). VFOXC1 classification identified 460 (65.2%) patients with BLBC (VFOXC1 ≥4) and 245 (34.8%) with non-BLBC (VFOXC1 <4). The distribution of patients with BLBC and non-BLBC by VFOXC1 score was not associated with any clinicopathologic characteristics of the cohort (χ^2 test $P > 0.05$; **Table 1**). With regard to standard Ki67 IHC assessment, high Ki67 (≥20%) correlated with VFOXC1 BLBC (≥4) cases, and low Ki67 (<20%) correlated with VFOXC1 non-BLBC (<4) TNBC tumors (*P* value <0.0001; Supplementary Table S2).

FOXC1 expression association with BLBC/non-BLBC category variables

ROC analysis revealed a stronger association between VFOXC1 expression and PAM50 subtyping (AUC = 0.87; 95% CI, 0.84–0.91) compared with the surrogate IHC phenotype for BLBC identification (AUC = 0.55; 95% CI, 0.50–0.60; Supplementary Table S3). The interassay reliability assessment, measured with the κ index, showed moderate agreement between PAM50 intrinsic subtyping and VFOXC1 expression (κ index 0.43; $P < 0.0001$), whereas agreement between the IHC-based BLBC/non-BLBC subtyping and PAM50 was minimal to slight (κ index 0.11; $P = 0.007$; Supplementary Table S4). Consequently, these findings indicated that VFOXC1 expression was reasonably consistent with the gold-standard PAM50 intrinsic subtyping, highlighting its reliability in approximating PAM50-defined BLBC within TNBC.

TNBC subtyping by VFOXC1 to predict capecitabine-based adjuvant treatment benefit

BLBC subtyping by VFOXC1 in TNBC was not associated with DRFS in the entire cohort (HR, 0.94; 95% CI, 0.68–1.30; $P = 0.713$). Nevertheless, VFOXC1 identified a subset of patients with non-BLBC TNBC who showed a significant improvement in DRFS when treated with capecitabine. In the capecitabine arm, univariate analysis revealed a notable benefit for this subset (interaction $P = 0.117$; HR, 0.53; 95% CI, 0.31–0.90; $P = 0.019$; **Fig. 2B**), confirming the IHC subtype subset analysis conducted during the clinical trial, as well as the PAM50 subtyping results from the prior GEICAM_CIBOMA translational analysis (10). Conversely, the VFOXC1 BLBC subset of patients did not show significant differences in the benefit of the trial's treatment options (**Fig. 2C**).

In multivariate analysis, adjusting for multiple testing, the VFOXC1 non-BLBC subtype emerged as a strong independent predictor of capecitabine benefit for DRFS (HR, 0.44; 95% CI, 0.25–0.76; $P = 0.003$) when compared with the VFOXC1 BLBC subtype (HR, 0.82; 95% CI, 0.55–1.22; $P = 0.323$; **Fig. 3A**). As previously demonstrated (10), the PAM50 non-BLBC subtype maintained a higher predictive value for capecitabine benefit in the multivariate DRFS analysis (interaction $P = 0.025$; HR, 0.17; 95% CI, 0.06–0.50; $P = 0.002$; **Fig. 3A**). The 5-year and 8-year DRFS multivariate analyses further confirmed the significant predictive value of VFOXC1 non-BLBC status in this TNBC cohort (**Fig. 3B** and **C**). These findings suggest that VFOXC1, when used as a single biomarker for BLBC subtyping, can independently identify patients with TNBC who are most likely to benefit from capecitabine following (neo)adjuvant CT. Given the demonstrated association between VFOXC1 expression and the PAM50-defined BLBC subtype (Supplementary Table S4), these results further confirm the ability of VFOXC1 to predict capecitabine efficacy in patients with non-BLBC TNBC (**Fig. 3**). Notably, the predictive value of VFOXC1 non-BLBC was independent of Ki67 proliferation status (Supplementary Table S5). The VFOXC1 non-BLBC subtype did not show any association with DRFS in the control arm.

In the secondary analysis focusing on DFS, a clear trend emerged: Patients classified as non-BLBC by VFOXC1 expression exhibited superior capecitabine efficacy compared with those classified as BLBC (Supplementary Fig. S1). The multivariate DFS-adjusted model confirmed this predictive value of VFOXC1 non-BLBC subtype (HR, 0.47; 95% CI, 0.28–0.78; $P = 0.003$), outperforming both gold-standard PAM50 and IHC phenotype non-BLBC subtyping in accurately identifying patients likely to respond favorably to capecitabine (Supplementary Table S6). Notably, the significant predictive power of VFOXC1 extended beyond DFS, also influencing OS analysis, further supporting its potential as a valuable biomarker for treatment decision-making in patients with non-BLBC TNBC (Supplementary Fig. S2; Supplementary Table S7).

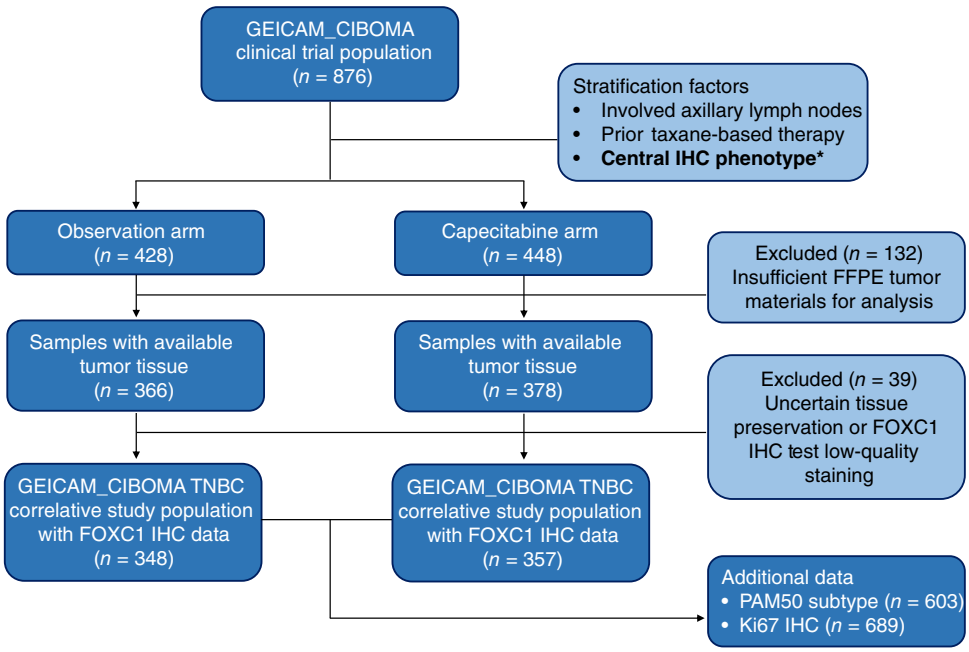

Figure 1.
CONSORT flow diagram for cases included in the GEICAM_CIBOMA translational study cohort of TNBC. The analysis of the translational study cohort followed a prospective–retrospective design testing prespecified primary and secondary hypotheses using high-quality clinical trial materials with adherence to REMARK criteria and to the guidelines for the use of archived clinical trial specimens for predictive biomarker evaluation on clinical trials. *IHC basal-like status was defined as TNBC with any staining for CK5/6 or EGFR.

Additional secondary endpoint RFS analysis demonstrated that patients with VFOXC1 non-BLBC retained their predictive value for capecitabine benefit, considering both distant and locoregional recurrence events in this TNBC cohort (multivariate analysis: HR, 0.39; 95% CI, 0.22–0.72; $P = 0.002$). Furthermore, the RFS analysis indicated that VFOXC1 BLBC/non-BLBC subtyping exhibited greater predictive power than PAM50 and IHC subtyping (Supplementary Fig. S3; Supplementary Table S8).

Patients with TNBC from the capecitabine treatment arm had lower distant recurrence events in the VFOXC1 non-BLBC subpopulation compared with the VFOXC1 BLBC subpopulation (9.5% vs. 15.3%, respectively). These data matched the distant recurrence event distribution of the entire GEICAM_CIBOMA trial cohort (9). Nevertheless, locoregional recurrence events in the VFOXC1 non-BLBC subpopulation were similar to those in the VFOXC1 BLBC subpopulation (0.8% vs. 0.9%, respectively; Supplementary Table S9).

Discussion

The current study presents a prespecified correlative analysis of high-quality tumor samples from the GEICAM_CIBOMA trial, investigating the potential of using the standardized FOXC1 IHC test for BLBC/non-BLBC subtyping in TNBC. Our analysis focused in evaluating the ability of FOXC1-based subtyping to predict the benefit of extended adjuvant capecitabine therapy. Our findings confirm that non-BLBC status, assessed by a single biomarker (VFOXC1), is a strong independent predictor of clinical benefit from capecitabine in patients with early-stage TNBC, offering a survival advantage compared with BLBC tumors.

The predictive capacity of the non-BLBC subtype for capecitabine benefit was previously observed in the GEICAM_CIBOMA trial by CK5/6 and EGFR IHC assessment (IHC phenotype), as part of a preplanned subgroup analysis (9). More recently, we reported that PAM50 non-BLBC was the most significant predictor of capecitabine benefit in TNBC, based on gene expression analysis of tumor specimens from the same trial. This multigene expression profiling enhanced the predictive information, reflecting the underlying biology of capecitabine response in these patient populations (10). Furthermore, the non-BLBC subtype was found to be the most significant predictor of capecitabine benefit, even when adjusting for metagene and 38 individual genes previously identified in the FinXX trial as predictive of capecitabine efficacy (29).

Although PAM50 intrinsic subtyping remains the gold standard for distinguishing BLBC and non-BLBC tumors, its high cost and limited accessibility prevent its routine use in clinical practice. In contrast, cost-effective semiquantitative IHC assays, which have been successfully standardized in breast cancer diagnosis (e.g., ER, PR, HER2, and Ki67), offer a more feasible alternative. Along with this clinical unmet need with regard to BLBC/non-BLBC identification to aid oncologists' decision-making, the standardized FOXC1 IHC test has consistently demonstrated a robust capacity to identify the BLBC subtype throughout TNBC cohorts, showing a meaningful degree of accuracy compared with PAM50-defined BLBC (15). Although this standardized FOXC1 IHC test shows promise as a predictive biomarker, it is not intended to replace the precision of PAM50 subtyping. Instead, FOXC1-based subtyping of TNBC tumors from the GEICAM_CIBOMA trial demonstrated a high concordance with PAM50 subtyping, outperforming IHC phenotype classification in predicting capecitabine benefit. Notably, the non-BLBC subset defined by VFOXC1 exhibited a significant and independent predictive value for DRFS, the primary endpoint of this prespecified analysis, and OS in secondary analysis. In contrast, PAM50 intrinsic subtyping did not demonstrate OS predictive value in the same trial, as previously reported, reinforcing the potential of FOXC1 as a reliable biomarker for identifying patients with TNBC who are most likely to benefit from capecitabine therapy (10).

Beyond the consideration of distant recurrence events based on the primary endpoint DRFS, the predictive value of VFOXC1 for capecitabine benefit was further supported by the secondary endpoint RFS, which captures both distant and local/regional

Table 1. Patient and baseline characteristics of the GEICAM_CIBOMA translational study cohort according to treatment arm.

Characteristic	Observation arm (*n* = 348), *n* (%)	Capecitabine arm (*n* = 357), *n* (%)
Median age, years (range)	48.5 (23.0–82.0)	51.0 (20.0–79.0)
Region		
Spain	228 (65.5%)	232 (65.0%)
Latin America	120 (34.5%)	125 (35.0%)
Race		
White	263 (75.6%)	265 (74.2%)
Hispanic	68 (19.5%)	72 (20.2%)
African American	7 (2.0%)	12 (3.4%)
Other	10 (2.9%)	8 (2.2%)
Karnofsky performance status		
80	16 (4.6%)	5 (1.4%)
90	54 (15.5%)	48 (13.4%)
100	278 (79.9%)	304 (85.2%)
Menopausal status at diagnosis		
Postmenopausal	227 (65.2%)	255 (71.4%)
Premenopausal	121 (34.8%)	102 (28.6%)
Histologic type		
Infiltrating ductal carcinoma	299 (85.9%)	311 (87.1%)
Infiltrating lobular carcinoma	8 (2.3%)	8 (2.2%)
Infiltrating carcinoma NOS	41 (11.8%)	38 (10.7%)
Histologic grade		
G1	8 (2.3%)	12 (3.4%)
G2	60 (17.2%)	63 (17.6%)
G3	254 (73.0%)	266 (74.5%)
GX	26 (7.5%)	16 (4.5%)
Tumor size, cm		
≤2	113 (32.5%)	110 (30.8%)
>2 and ≤5	198 (56.9%)	214 (59.9%)
>5	37 (10.6%)	33 (9.3%)
Stage at diagnosis		
I	69 (19.8%)	63 (17.7%)
II	217 (62.4%)	225 (63.0%)
III	62 (17.8%)	69 (19.3%)
Nodal status		
Negative	192 (55.2%)	198 (55.5%)
1–3	103 (29.6%)	101 (28.3%)
≥4	53 (15.2%)	58 (16.2%)
Type of CT prior to randomization		
Adjuvant	300 (86.2%)	300 (84.0%)
Neoadjuvant	48 (13.8%)	54 (15.1%)
Unknown	0 (0%)	3 (0.9%)
pCR in patients with neoadjuvant CT		
No	35 (72.9%)	44 (81.5%)
Yes	13 (27.1%)	10 (18.5%)
CT regimen		
Anthracyclines and taxanes	236 (67.8%)	236 (66.1%)
Anthracyclines without taxanes	112 (32.2%)	121 (33.9%)
Breast surgery		
Conservative	201 (57.8%)	195 (54.6%)
Mastectomy	147 (42.2%)	159 (44.5%)
Unknown	0 (0%)	3 (0.9%)
Axillary surgery		
ALND ± SLNB	246 (70.7%)	269 (75.3 %)
SLNB	102 (29.3%)	88 (24.7%)
Radiotherapy		
No	281 (80.8%)	279 (78.2%)
Yes	67 (19.2%)	75 (21.0%)
Unknown	0 (0%)	3 (0.8%)
Distant relapse events		
No	277 (79.6%)	294 (82.4%)
Yes	71 (20.4%)	63 (17.6%)
Recurrence events		
No	248 (71.3%)	280 (78.4%)
Yes	100 (28.7%)	77 (21.6%)
Death events		
No	289 (83.1%)	306 (85.7%)
Yes	59 (16.9%)	51 (14.3%)
Ki67 IHC assessment		
Median Ki67, % (range)	21.8 (0–79.9)	21.5 (0–85.3)
Ki67 < 20%	158 (45.4%)	164 (45.9%)
Ki67 ≥ 20%	183 (52.6%)	184 (51.6%)
Unknown	7 (2.0%)	9 (2.5%)
PAM50 intrinsic subtype		
PAM50 non-BLBC	41 (11.8%)	51 (14.3%)
PAM50 BLBC	256 (73.5%)	255 (71.4%)
Unknown	51 (14.7%)	51 (14.3%)
IHC phenotype		
IHC non-BLBC	88 (25.3%)	90 (25.2%)
IHC BLBC[a]	260 (74.7%)	267 (74.8%)
VFOXC1		
<4 (non-BLBC)	117 (33.6%)	128 (35.9%)
≥4 (BLBC)	231 (66.4%)	229 (64.1%)

Abbreviations: ALND, axillary lymph node dissection; NOS, non otherwise specified; pCR, pathologic complete response; SLNB, sentinel lymph node biopsy.
[a]IHC BLBC phenotype: CK5/6-positive and/or EGFR-positive.

recurrence events. Among the secondary endpoints, RFS was the only measure that demonstrated a significant interaction between VFOXC1 and the trial's treatment arm categorization (interaction $P = 0.026$), whereas this significant RFS interaction was not observed for PAM50 BLBC subtyping (interaction $P = 0.395$; Supplementary Fig. S3). Given that high-risk early TNBC frequently recurs locoregionally, leading to substantial morbidity, these predictive findings from the RFS clinical endpoint further support FOXC1 as a promising predictive biomarker in these patients.

The findings from this study align with similar evidence from the ECOG-ACRIN EA1131 trial, which also demonstrated superior invasive DFS for patients with PAM50 non-BLBC treated with capecitabine compared with a platinum-based therapy (11). Together, these results underscore the potential clinical utility of identifying the non-BLBC subtype as a predictor of capecitabine efficacy in early-stage TNBC. Given the promising performance of VFOXC1 as a predictive biomarker, its use in clinical practice could enable more precise patient selection for capecitabine treatment, ultimately improving treatment outcomes.

Strengths of our study include the use of high-quality clinical trial materials, adherence to REMARK guidelines (21), and a formal design testing prespecified hypotheses for primary and secondary endpoints (20). The large sample size of the GEICAM_CIBOMA trial further strengthens the evidence supporting the predictive

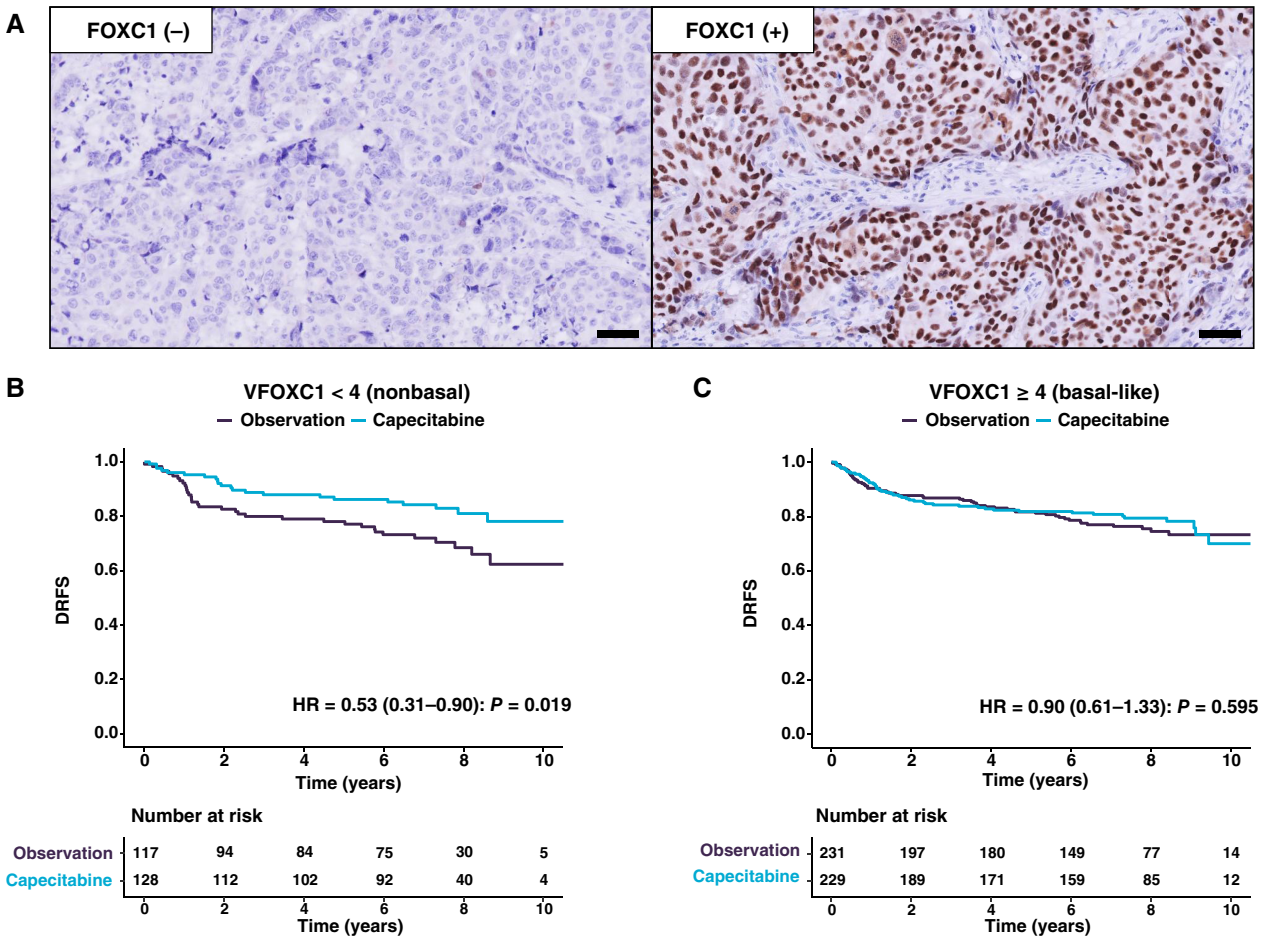

Figure 2.

Survival analyses showing the primary endpoint of DRFS for patients randomly assigned to the capecitabine or observation arm in the GEICAM_CIBOMA translational study cohort. **A,** FOXC1 IHC detection by the standardized FOXC1 IHC test, showing positive (right image) and negative (left image) staining in two representative TNBC tumor samples from the GEICAM_CIBOMA trial cohort. The line shows 30 μm. Magnification, ×200. **B and C,** Kaplan–Meier curves for VFOXC1.

ability of FOXC1 IHC for phenotyping non-BLBC tumors. This provides a strong justification for incorporating this biomarker in future clinical trials to select patients most likely to benefit from adjuvant capecitabine therapy. However, to achieve level 1B evidence, confirmation through additional prospective–retrospective clinical trial series is still necessary (20).

Notably, only a small proportion of patients with stage I TNBC were included in this cohort (132 patients, 19%), highlighting the need for further FOXC1 IHC assessment in additional TNBC cohorts with a higher representation of stage I cases. Capecitabine-based (neo)adjuvant treatment benefit in patients with stage I TNBC is an unmet clinical need compared with other clinical standard treatment options (30). Additional limitations of this translational study include the low proportion of patients who received neoadjuvant treatment (102 patients, 14%; 22% of them had pathologic complete response) and the limited diversity of CT regimens, with 67% of patients treated with anthracyclines plus taxanes, 33% with anthracyclines alone, and no patients receiving platinum-based combinations (**Table 1**).

For patients with early-stage TNBC with residual disease, predictive biomarkers are essential to improve long-term clinical outcomes. The CREATE-X trial demonstrated improved OS following 6 to 8 cycles of adjuvant capecitabine in patients with TNBC with residual disease after neoadjuvant CT (6). Similarly, the GEICAM_CIBOMA trial showed benefit from capecitabine in a subset of patients with nonbasal TNBC following neoadjuvant therapy based on IHC [by EGFR and CK5/6 lack of IHC staining (9)] and PAM50 subtyping (10). Moreover, ongoing clinical trials, such as the ECOG-ACRIN EA1131 (11), the randomized, multicenter phase II ShandongCHI-07 (NCT03703427), and phase II APOLLO (NCT04501523; ref. 31) trials, are exploring capecitabine-based therapies in patients with residual disease after neoadjuvant CT. Two ongoing phase III trials in patients with TNBC with residual disease after neoadjuvant therapy, ASCENT-05 (NCT05633654) and TROPION-Breast03 (NCT05629585), are comparing antibody–drug conjugates–based treatment with physician's choice regimens, including capecitabine. Incorporating FOXC1-based subtyping into these trials could enhance

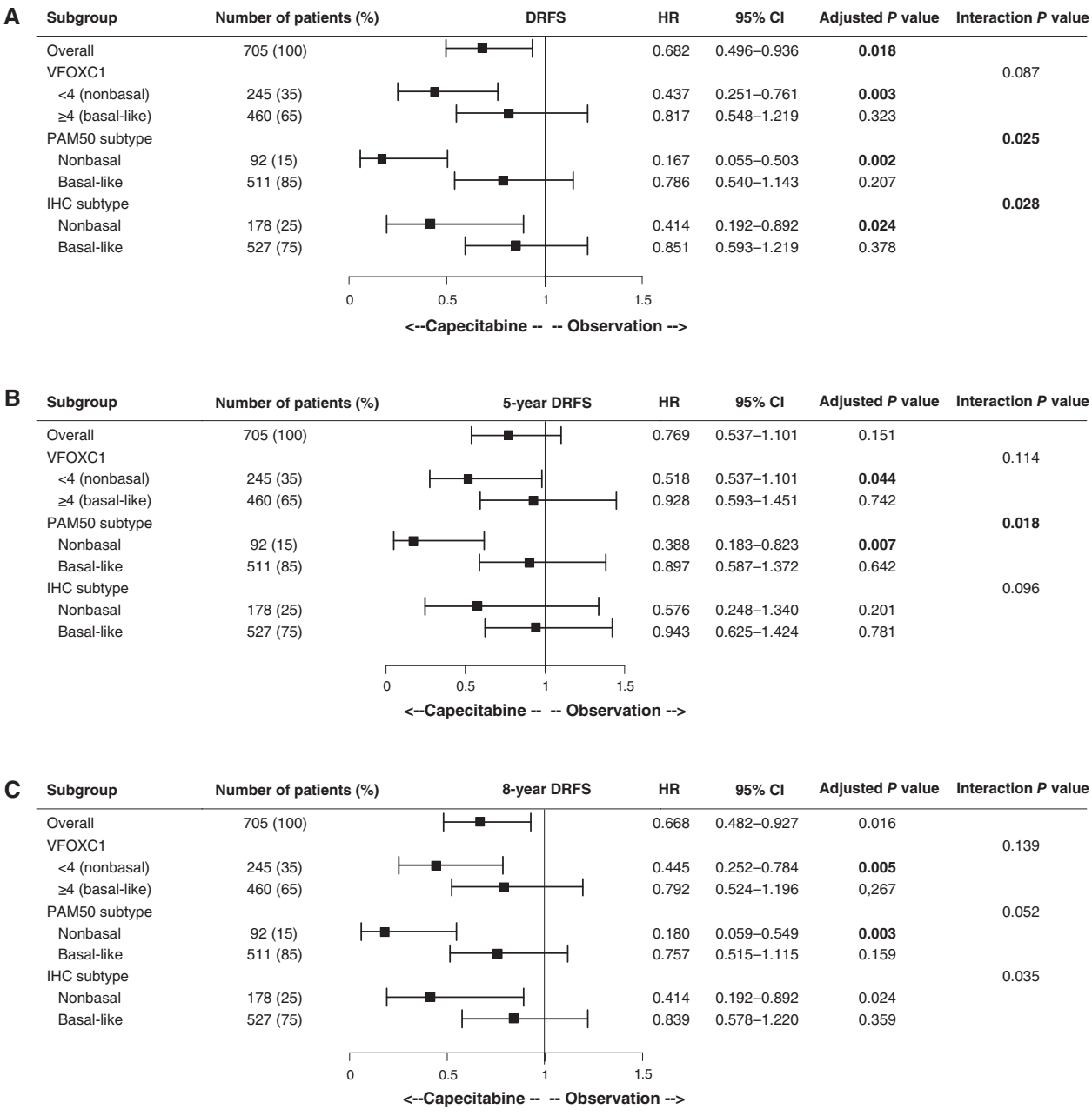

Figure 3.

Forest plot for the GEICAM_CIBOMA translational study cohort primary endpoint of DRFS at (**A**) full GEICAM_CIBOMA trial follow-up, (**B**) 5-year DRFS, and (**C**) 8-year DRFS on the capecitabine arm versus observation arm for basal-like/nonbasal category variables. HRs, 95% CI, and *P* values are derived from Cox regression analyses adjusted for age, menopausal status, histologic grade, tumor size, stage, breast surgery, region, nodal status, and CT regimen.

patient selection, helping to identify those most likely to benefit from capecitabine.

As we move toward personalized treatment strategies, FOXC1 offers a feasible and accessible method for non-BLBC identification in TNBC. This could aid in distinguishing between patients who will benefit from capecitabine therapy and those who may require alternative treatments. Ultimately, the development and validation of biomarkers are crucial to optimizing therapeutic approaches for this high-risk population.

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Authors' Contributions

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Note

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