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^{18}F-FDG-PET-guided, pathological complete response-adapted chemotherapy de-escalation in HER2-positive early breast cancer: 5-year outcomes and exploratory ctDNA analyses from the randomized phase 2 PHERGain trial

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Abstract

PHERGain is a randomized, non-comparative phase 2 trial evaluating an 18F-FDG-PET–guided, pathological complete response (pCR)-adapted strategy to omit chemotherapy in HER2-positive early breast cancer (EBC). Patients were randomized to receive chemotherapy plus trastuzumab and pertuzumab (HP; group A) or HP ± endocrine therapy (ET; group B). In group B, treatment was adapted according to PET response after two cycles and pCR, with chemotherapy administered to non-responders or patients without pCR. After a median follow-up of 5.6 years, 5-year invasive disease-free survival (iDFS) was 89.5% (95% CI, 85.7–93.4) in group B and 92.4% (95% CI, 86.7–98.5) among PET responders achieving pCR without chemotherapy, with one distant recurrence. Detectable ctDNA at baseline or during treatment was associated with inferior iDFS, whereas ctDNA clearance correlated with higher pCR rates and improved long-term outcomes. These findings continue to support the clinical validity of a PET-guided, pCR-adapted approach and suggest a potential role for ctDNA in refining treatment individualization.

Introduction

Human epidermal growth factor receptor 2–positive (HER2+) breast cancer constitutes a unique and molecularly heterogeneous breast cancer subtype, characterized by amplification of the *ERBB2*/*HER2* gene and/or overexpression of the HER2 protein, and accounts for approximately 15–20% of all cases[1,2]. This tumor subtype has presented substantial outcome improvements since the implementation of HER2-targeted therapies[3–6]. These therapeutic advances have facilitated the design of de-escalation strategies in selected subgroups of patients with HER2+ early breast cancer (EBC). The neoadjuvant setting provides an ideal context for this approach, as pathological complete response (pCR) is a favorable prognostic factor at the individual level for long-term outcomes, including disease-free survival (DFS) and overall survival (OS)[7].

The PHERGain study (NCT03161353) showed the feasibility of a ^{18}F-fluorodeoxyglucose-positron emission tomography (^{18}F-FDG-PET)–guided, pCR-adapted strategy to avoid chemotherapy in patients with HER2+ EBC undergoing neoadjuvant dual HER2 blockade with trastuzumab and pertuzumab. The first primary endpoint, pCR, demonstrated that 37.9% of patients in the adaptive treatment arm (group B) who were classified as ^{18}F-FDG-PET responders after two initial cycles of trastuzumab and pertuzumab and continued with six additional cycles of the same chemotherapy-free regimen, obtained a pCR at surgery[8].

At a median follow-up of three years, the second primary endpoint—3-year invasive disease-free survival (iDFS)—revealed that 94.3% (95% CI: 90.9 to 96.8) of patients who followed this strategy remained cancer-free, even though chemotherapy was spared in nearly one-third of patients. Importantly, group B patients who were ^{18}F-FDG-PET responders and reached a pCR with trastuzumab and pertuzumab alone,

without ever receiving chemotherapy, also achieved outstanding 3-year outcomes, with no cases of metastatic relapse[9].

Recurrence patterns and survival outcomes vary according to hormone receptor status in HER2+ EBC, likely reflecting differences in the distribution of PAM50 intrinsic subtypes. Specifically, hormone receptor-positive (HR+) tumors are enriched for PAM50 luminal subtypes, whereas hormone receptor-negative (HR-) tumors are more frequently classified as PAM50 HER2-enriched[10]. As a result, patients with HR- tumors show a lower incidence of bone metastases but a higher mortality rate within the first five years compared with those with HR+ disease[11]. Notably, a considerable proportion of late recurrences has been documented among HER2+ EBC patients with HR+ tumors[12,13], who represent two thirds of the PHERGain population. Therefore, extended follow-up is essential to enable a comprehensive characterization of long-term outcomes in this study.

Nonetheless, clinical endpoints alone may not fully capture the biological heterogeneity underlying long-term responses. Liquid biopsy-based approaches, particularly the detection of circulating tumor deoxyribonucleic acid (ctDNA), now allow sensitive monitoring of minimal residual disease (MRD)[14]. ctDNA has been proved as an emerging tool for risk stratification and real-time assessment of treatment response in EBC[15–17] and may improve the prediction of pCR and long-term outcomes, offering the potential to personalize treatment decisions.

In this planned analysis of the PHERGain trial, we first report the 5-year iDFS among patients included in groups A and B who underwent surgery according to protocol together with additional secondary efficacy endpoints. We then assess a tissue-free epigenomic-based ctDNA assay (Guardant Reveal^{TM})for MRD detection to improve the prediction of pCR and 5-year outcomes, thereby supporting more tailored (neo)adjuvant treatment strategies for HER2+ EBC patients within the PHERGain framework.

Results

Patient disposition and follow-up

Patients were recruited from 45 centers in seven countries between June 26, 2017, and April 24, 2019. Of the 376 enrolled patients, 71 (18.9%) were assigned to group A and 285 (75.8%) were included in group B. An additional 20 (5.3%) of 376 patients with subclinical metastatic disease by ^{18}F-FDG-PET were assigned to an exploratory group C and are not reported in this manuscript (**Supplementary Fig. 1**).

A total of 68 (95.8%) patients in group A and 283 (99.3%) patients in group B received at least one trial drug; no patients are still receiving treatment. Among all randomized patients, 63 (89.0%) and 267 (93.7%) patients proceeded to surgery according to protocol in groups A and B, respectively. The reasons for not starting study treatment and not performing surgery according to protocol have been already reported[9] (**Fig. 1**).

Of the 55 patients in group A and 250 patients in group B who completed the initial 3-year follow-up, 49 (89.1%) and 215 (86.0%), respectively, provided re-consent to participate in the extension phase (**Fig. 1**). At this updated analysis (data cut-off, November 4, 2024), the median duration of follow-up was 5.6 years (range, 0.4 to 7.3). Patient baseline characteristics were previously described[8,9].

5-year efficacy outcomes

In group B, a total of 26 (9.7%) of 267 patients who underwent surgery according to protocol had an invasive disease event or death within 5 years after surgery. The estimated 5-year iDFS rate for the group B ITT population was 89.5% (95% confidence interval [CI], 85.7 to 93.4) **(Fig. 2)**. The majority of iDFS events consisted of distant recurrences (5.2%) and second primary non-breast cancer (1.5%). A detailed description of iDFS events is provided in **Table 1**. Of interest, among group B patients who were [^{18}F]FDG-PET responders, achieved a pCR, and did not receive chemotherapy during the study treatment (n = 86), the 5-year iDFS rate was 92.4% (95% CI, 86.7 to 98.5) with six iDFS events overall, including one (1.2%) patient with distant recurrence. The estimated 5-year distant disease-free survival (DDFS) rate for patients assigned to group B and group B responders were 92.7% (89.5 to 96.0) and 98.7% (96.3 to 100.0), respectively (**Table 1**).

Among 63 patients included in group A that underwent surgery according to protocol, the estimated 5-year iDFS, DFS, and DDFS rates were 96.7% (95% CI, 92.2 to 100), 96.7 (95% CI, 92.2 to 100), and 96.7% (95% CI, 92.2 to 100), respectively. In the overall randomized population, the estimated 5-year OS rate was 98.4% (95% CI, 95.3 to 100.0) for patients assigned to group A and 98.1% (95% CI, 96.5 to 99.8) for patients assigned to group B. Deaths occurred in one (1.4%) patient in group A (due to distant recurrence event) and five (1.8%) patients in group B (four due to distant recurrences events and one by suicide) within 5 years after surgery **(Table 1)**.

ctDNA analysis

Of the 356 patients included in groups A and B of the PHERGain study, 932 plasma samples were collected: 336 at baseline, 311 at C3D1, and 285 at the pre-surgical timepoint (**Supplementary Fig. 2**). Of these, 801 (81.0%) samples were successfully sequenced (288 at baseline, 270 at C3D1, and 243 at the pre-surgical timepoint). Samples that failed sequencing were predominately due to low cfDNA extractions below the assay recommended input. To investigate ctDNA dynamics, paired longitudinal analyses were performed in patients with available matched samples, including 235 baseline–C3D1 pairs to assess early ctDNA clearance and 210 baseline–pre-surgery pairs to evaluate late ctDNA clearance at the pre-surgical timepoint.

Baseline ctDNA detection and longitudinal dynamics during neoadjuvant therapy

ctDNA was detected in 204 (71.0%) of 288 patients prior to initiation of neoadjuvant therapy (**Fig 3A**). Baseline ctDNA detection was strongly associated with disease stage, increasing from 33.0% in stage I (9/27) to 93.2% in stage III disease (41/44) ($p<0.001$; **Fig. 3B**). Similarly, pre-treatment ctDNA detection

correlated with nodal status, with detectable ctDNA observed in 122 (87.8%) of 139 patients with lymph node involvement versus 82 (55.0%) of 149 patients without nodal involvement (p<0.001; **Fig. 3C**). Among patients with detectable ctDNA at baseline, 61 (48.4%) of 126 achieved early ctDNA clearance at C3D1, whereas 61 (49.2%) of 124 reached late ctDNA clearance at the pre-surgical timepoint (**Fig. 4**). Persistent ctDNA detection was observed in 24% and 12% of patients with detectable ctDNA at baseline after cycle 3 and at pre-surgery respectively. Notably, no patient with undetectable ctDNA at baseline developed detectable ctDNA during neoadjuvant therapy (**Fig. 4D**).

Association of baseline ctDNA and ctDNA clearance with pCR

Baseline ctDNA status was not significantly associated with pCR, as comparable pCR rates were reported in patients with detectable versus undetectable ctDNA (41.7% vs 38.1%; p=0.583; **Fig. 3D**). In contrast, early ctDNA clearance at C3D1 was associated with higher pCR rates compared with persistent ctDNA detection (48.4% vs 22.0%; p=0.003; **Fig. 4B**). ctDNA clearance also strongly correlated with PET response, observed in 90.5% (114/126) of PET responders versus 56.1% (23/41) of non-responders (**Supplementary Fig. 3**). Likewise, late ctDNA clearance at the pre-surgical timepoint was strongly associated with achieving pCR (49.2% vs 0.0%; p<0.001; **Fig. 4D**). Therefore, none of the patients with detectable ctDNA pre-surgery (n=28) achieved a pCR.

Prognostic impact of ctDNA dynamics on 5-year outcomes

Detectable ctDNA at baseline in all patients identified patients at higher risk of invasive recurrence, with inferior 5-year iDFS compared with those with undetectable ctDNA (86.7% vs 97.1%; Hazard ratio [HR] HR 3.1, 95% CI 1.0 to 9.8; p=0.021; **Fig. 5A**). Similarly, ctDNA positivity at C3D1 and pre-surgery was associated with worse 5-year iDFS (77.5% vs 94.4%; HR 4.3, 95% CI 1.8 to 9.9; p=0.011; **Fig. 5B** and 81.1% vs 94.0%; HR 3.4, 95% CI 1.2 to 9.3; p=0.0281; **Fig. 5C**, respectively).

In multivariable Cox regression analyses of ctDNA clearance (reference) vs no ctDNA clearance adjusting for disease stage and nodal status, detectable ctDNA remained an independent predictor of iDFS at baseline (HR 2.9, 95% CI 0.9 to 9.6; p=0.043) and at C3D1 (HR 3.6, 95% CI 1.4 to 8.4; p=0.006), but not at pre-surgery (HR 2.4, 95% CI 0.8-8.2; p=0.1205).

Early ctDNA clearance at C3D1 was associated with improved 5-year iDFS compared with persistent ctDNA detection (92.3% vs 75.6%; HR 2.9, 95% CI 1.1 to 7.3; p=0.028; **Fig. 5C**). This association remained evident in multivariate Cox regression analyses adjusting for disease stage and nodal status, although the effect did not reach statistical significance (HR 2.4, 95% CI 0.9 to 6.3; p=0.072). Late pre-surgical ctDNA dynamics were not significantly associated with 5-year iDFS (91.4% vs 78.9%; p=0.075), nor did they retain significance in multivariable Cox regression analysis (HR 2.0, 95% CI 0.6–6.0; p=0.237). The associations between ctDNA status at baseline, C3D1 and pre-surgery, as well as early and late ctDNA clearance, and DDFS are present in **Supplementary Fig. 4**.

Discussion

The combination of neoadjuvant chemotherapy with dual HER2 blockade using trastuzumab and pertuzumab has become the treatment of choice for patients with stage II/III HER2+ breast cancer. This approach consistently achieves high percentage of pCR, which strongly associates with favorable long-term outcomes, while increasing evidence supports the exploration of less intensive treatment strategies for selected patients. On this basis, the PHERGain trial investigated a novel, individualized de-escalation strategy guided by early [^{18}F]FDG-PET assessment and pCR, based on dual HER2 blockade with trastuzumab and pertuzumab as the therapeutic backbone.

At 5 years post-surgery, patients in group B managed with this [^{18}F]FDG-PET-based, pCR-adapted approach achieved an iDFS rate of 89.5% (95% CI, 85.7 to 93.4), despite nearly one-third never received chemotherapy and the rest initiated it later during study treatment. This reflects a delta increase of 5.3 percentage points in new invasive disease events after 2 additional years of follow-up, consistent with the progressive occurrence of recurrences observed in HER2+ EBC and the gradual decline in efficacy endpoints reported in most pivotal trials in this setting.

Although PHERGain included randomized stratification, it was not designed for a formal head-to-head comparison between groups A and B. The 1:4 randomization resulted in a smaller control arm, limiting statistical precision; group A primarily served as an internal benchmark rather than a hypothesis-testing comparator. At 5 years, iDFS in group A was 96.7% (95% CI, 92.2 to 100.0), numerically higher than in group B, with no additional invasive events during extended follow-up. However, this observation should be interpreted with caution in view of the previously noted limitations.

Other indirect cross-trial comparisons should also be interpreted with caution, given the heterogeneity of study populations, differences in endpoints, and the absence of mature long-term data in several trials. Indeed, although 5-year iDFS data have not been specifically reported in most of these studies, other 5-year outcomes reported in PHERGain seem to align closely with results from other trials evaluating neoadjuvant chemotherapy combined with dual HER2 blockade in HER2+ EBC. In NeoSphere, the 5-year DFS was 84.0% (95% CI, 72.0 to 91.0); in BERENICE, 5-year event-free survival (EFS) reached 90.8% (95% CI, 86.5 to 95.2) and 89.2% (95% CI, 84. to–93.6) across anthracycline- and taxane-based backbones[18]; and in PEONY, 5-year DFS and EFS were 86.0% and 84.8% (95% CI not reported), respectively[19].These data are consistent with the 89.5% (95% CI, 85.7 to 93.4) observed in our study, despite the fact that one third of patients did not receive chemotherapy.

Notably, when PHERGain and the other aforementioned trials were initiated, trastuzumab emtansine (T-DM1) had not yet been approved for patients with residual disease after neoadjuvant therapy, a population in whom this agent has subsequently demonstrated significant improvements in both iDFS and OS. Accordingly, incorporating T-DM1 into these study protocols would likely have conferred additional survival benefits. Reflecting this evolution, the ongoing PHERGain-2 trial has incorporated T-DM1, thereby addressing the unmet need of patients with residual disease following neoadjuvant therapy and aligning the study with contemporary standards of care in HER2+ EBC.

Therapeutic de-escalation has moved from concept to reality in HER2+ EBC, largely enabled by the predictive value of the neoadjuvant approach. pCR has emerged as a validated surrogate biomarker of long-term prognosis and provides a reliable framework for tailoring therapy intensity. Currently, three main strategies for de-escalation are being explored: (i) reducing the intensity of chemotherapy, (ii) shortening the number of chemotherapy cycles given alongside trastuzumab and pertuzumab, and (iii) omitting chemotherapy altogether in selected patients, treating them exclusively with anti-HER2 therapy.

The first step in reducing chemotherapy intensity with dual HER2 blockade was demonstrating anthracyclines were not required in HER2+ EBC. TRYPHAENA[20] and TRAIN-2[19] confirmed platinum-taxane regimens achieved similar pCR and outcomes as anthracycline- and taxane-based therapy. More recently, HELEN-006[22] and neoCARHP[23] further suggested platinum-taxane yields pCR rates comparable to single-agent taxane. While survival results are pending, these data seem to support single-agent taxane plus trastuzumab and pertuzumab as a valid neoadjuvant alternative for a subgroup of patients.

Reducing chemotherapy cycles within a trastuzumab- and pertuzumab-based regimen represents a second de-escalation approach. In TRAIN-3[24], advanced imaging identified early responders eligible for surgery after only three cycles of chemotherapy plus dual HER2 blockade. Those achieving early pCR sustained excellent outcomes, with breast magnetic resonance imaging showing the highest concordance between radiological and pathological response, particularly in hormone receptor-negative tumors[25].

Finally, the most ambitious de-escalation strategy involves complete omission of chemotherapy. In PHERGain, an [^{18}F]FDG-PET–guided, pCR-adapted approach enabled nearly one-third of patients with HER2-positive early breast cancer to avoid chemotherapy while maintaining durable disease control and improved tolerability. Notably, among PET responders achieving pCR with trastuzumab and pertuzumab alone, only one distant recurrence was observed at 5 years. PHERGain-2 is further exploring a chemotherapy-free strategy in selected patients with small, node-negative, HER2 IHC 3+ tumors treated with dual HER2 blockade alone; patients without pCR receive adjuvant T-DM1. Although efficacy outcomes are pending, these studies support the feasibility of chemotherapy-free approaches and highlight neoadjuvant response as a key biomarker for treatment individualization. Whether such strategies should extend to stage II/III disease or remain limited to stage I tumors remains an open question.

Both PHERGain and TRAIN-3 also reinforce the concept that pCR retains its prognostic significance regardless of the treatment regimen, as long as anti-HER2 therapy is included. This contrasts with earlier observations, such as those from the NOAH trial[26], which suggested that patients achieving pCR with chemotherapy plus trastuzumab had better outcomes than those achieving pCR with chemotherapy alone.

While this de-escalation approach continues to confirm excel results, the relationship between long-term efficacy outcomes and underlying molecular mechanism remains unclear. Recent reports in EBC have

shown that ctDNA can be tracked during neoadjuvant therapy as a predictive tool for response and outcome[15,17,27]. In this study, we explored whether ctDNA detection and its early and late dynamics during neoadjuvant therapy could predict pCR and 5-year outcomes. ctDNA detection and its longitudinal dynamics provided clinically relevant prognostic and predictive information in this setting. Early and late ctDNA clearance during neoadjuvant therapy were associated with higher pCR rates and improved 5-year outcomes. Conversely, detectable ctDNA at any timepoint, as well as the absence of early or late ctDNA clearance, consistently identified patients at increased risk of recurrence and inferior 5-year outcomes. These findings suggest that ctDNA monitoring may complement imaging- and pathology-based response assessment, refining risk stratification and contributing to a more individualized therapeutic approach in HER2+ EBC.

The limitations of PHERGain have been already described and include its non-comparative phase II design, modest sample size, the use of [^{18}F]FDG-PET to guide treatment, which may limit generalizability , the lack of adjuvant T-DM1 in patients without pCR, and the relatively short initial follow-up[28]. Although the present analysis has extended follow-up, it also carries inherent limitations that warrant consideration. First, a substantial proportion of patients did not provide re-consent for participation in this extension phase and were therefore censored, which may have introduced attrition bias and reduced the robustness of long-term outcome estimates. Second, the follow-up assessments were relatively limited in scope, relying on simple clinical evaluations rather than comprehensive imaging or biomarker-based monitoring. Together, these factors may have led to underdetection of subclinical events and restricted the ability to fully characterize the durability of benefit associated with the chemotherapy-free approach. Additionally, an important limitation is the adaptative nature of the study design, whereby treatment allocation in group B was modified according to early treatment response; therefore, ctDNA findings should be interpreted with caution and considered exploratory in nature.

In conclusion, this strategy-based, randomized, non-comparative, multicenter, open-label phase 2 study, with 5 years of follow-up, continues to demonstrate that an [^{18}F]FDG-PET-based, pCR-adapted strategy can identify a subset of HER2+ EBC patients who may safely omit chemotherapy and be treated exclusively with dual HER2 blockade using trastuzumab and pertuzumab. A critical next step is the precise identification of patient subgroups most likely to derive durable benefit from this chemotherapy-free strategy through advanced predictive biomarkers. In this context, ctDNA detection and longitudinal dynamics represent a promising tool for response assessment and MRD evaluation, enabling improved risk stratification in HER2+ EBC and potentially guiding the design of future therapeutic escalation and de-escalation trials. Meanwhile, selected patients may derive benefit from this therapeutic approach, potentially avoiding the need for chemotherapy.

Methods

Study design and participants

PHERGain is a strategy-based, multicenter, randomized, non-comparative, open-label phase 2 study in patients with HER2+ operable breast cancer. The trial protocol is available in the Supplementary Information.

Details of the trial design and study population have been already reported[9,29]. Patients were recruited from 45 hospitals in seven European countries (Spain, France, Belgium, Germany, UK, Italy, and Portugal). Females ≥18 years of age who had the following characteristics were enrolled: previously untreated, centrally confirmed HER2+, stage I-IIIA, invasive, operable breast cancer (≥1.5 cm tumor size), with at least one breast lesion evaluable by ^{18}F-FDG-PET. Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, a left ventricular ejection fraction Q≥55%, and adequate organ function were also required. The main exclusion criteria were metastatic disease identified by standard staging evaluation), bilateral breast cancer, or previous treatment for this invasive breast cancer. Full eligibility criteria are listed in the trial protocol, which is available with the full text of this article in **Supplementary Methods p.1-2**.

In August 2022, the protocol was amended to extend follow-up of PHERGain participants, with long-term outcomes planned at 5 and 7 years after surgery. Patients provided new informed consent to participate in this extension phase, which was reviewed and approved by the corresponding ethics committees.

Procedures

An overview of the study design is provided in **Supplementary Methods and Supplementary Fig. 1**. All study drugs were administered intravenously, with the exception of trastuzumab, which was given subcutaneously at a fixed dose of 600 mg. Drugs were administered every three weeks. The pertuzumab loading dose was 840 mg, followed by a maintenance dose of 420 mg. Docetaxel was administered at 75 mg/m^2. Carboplatin was administered at a dose of AUC6 (area under the curve, 6 mg/mL/min). Dose modifications were not permitted for trastuzumab and pertuzumab. Dose reductions of docetaxel and carboplatin were allowed, as per local prescribing information.

According to the study protocol, ^{18}F-FDG-PET was performed before randomization and after two cycles of neoadjuvant therapy in groups A and B. Patients allocated to group A received six cycles of docetaxel and carboplatin concurrently with trastuzumab and pertuzumab, regardless of the on-treatment ^{18}F-FDG-PET results. All patients enrolled in group B initially received two cycles of trastuzumab and pertuzumab. Hormone receptor–positive patients also received letrozole (2.5 mg/day orally) if they were post-menopausal or tamoxifen (20 mg/day orally) if they were pre- or peri-menopausal. Group B patients with ≥40% reduction of the maximum standardized uptake value (SUVmax) from the baseline to on-treatment ^{18}F-FDG-PET (group B/^{18}F-FDG-PET-responders) continued the same treatment for six more cycles (for a total of eight cycles of trastuzumab and pertuzumab, with or without endocrine therapy). Group B patients who did not achieve this reduction (group B/^{18}F-FDG-PET-non responders) switched to neoadjuvant chemotherapy consisting of six cycles of docetaxel and carboplatin plus concurrent trastuzumab and pertuzumab.

Patients who discontinued the neoadjuvant phase of the study treatment could start another neoadjuvant therapy or undergo surgery outside the clinical trial. Surgery (breast conservation or mastectomy with sentinel lymph node evaluation or axillary dissection) according to protocol was done 2-6 weeks after the last treatment cycle of the neoadjuvant phase.

After surgery, group B/^{18}F-FDG-PET-responders who did not achieve pCR received an additional six cycles of adjuvant docetaxel and carboplatin plus concurrent trastuzumab and pertuzumab. All patients from groups A and B completed up to 18 cycles of treatment with trastuzumab and pertuzumab in the absence of disease progression or unacceptable toxicity, patient withdrawal, or investigator's decision. Adjuvant endocrine therapy and radiotherapy were administered as per hormone receptor status and institutional practices, respectively.

During the extended follow-up phase of the study, which encompassed the period from 4 to 7 years after the last patient's surgery, participants underwent annual on-site visits including a physical examination and mammography. Additional imaging procedures were performed when clinically indicated and in accordance with institutional guidelines. Investigators made every effort to ensure the timely and accurate detection of invasive disease events, thereby maintaining the robustness and reliability of long-term outcome assessments.

Outcomes

The primary endpoints—pCR (ypT0/is ypN0) rate by local assessment among group B/^{18}F-FDG-PET responders, and 3-year iDFS in group B of the PHERGain study—have been previously reported[28,29]. Here, we provide an updated assessment of the second primary endpoint, 5-year iDFS in patients allocated to group B who underwent protocol-defined surgery, together with additional secondary efficacy endpoints (5-year iDFS among patients in group A and 5-year DFS, DDFS, and OS in patients assigned to groups A and B). We also evaluated the association between ctDNA, clinicopathological characteristics, pCR rates, and 5-year outcomes, a prespecified exploratory endpoint of PHERGain.

5-year iDFS was defined as the percentage of patients who were alive without ipsilateral invasive breast tumor recurrence, ipsilateral locoregional invasive breast cancer recurrence, distant recurrence, contralateral invasive breast cancer, second non-breast primary cancer, and death from any cause at 5 years after surgery.

5-year DDFS was defined as the percentage of patients who were alive without distant disease recurrence and death from any cause at 5 years after surgery.

5-year OS was defined as the percentage of patients who were alive at 5 years after randomization.

ctDNA analysis

ctDNA was analyzed in plasma samples collected at baseline, C3D1, and prior to surgery using Guardant Reveal^{TM} (Guardant Health Redwood City, CA), a tissue-free epigenomic assay that evaluates over

20,000 methylation regions to detect ctDNA[14,30]. The test analyzes plasma-derived cell-free DNA and identifies tumor-associated DNA methylation patterns without requiring prior tumor tissue sequencing. It employs a tumor-agnostic, methylation-based classifier trained on breast cancer–associated epigenomic features and interrogates approximately 3,000 differentially methylated genomic regions to classify each plasma sample as ctDNA detected or not detected.

ctDNA detection was assessed at each timepoint, and paired longitudinal analyses were performed to evaluate early ctDNA clearance at C3D1 and late clearance at the pre-surgical timepoint relative to baseline.

Statistical analysis

Details of the statistical design have been already published[28]. Efficacy analyses for 5-year outcomes were performed in the intention-to-treat population, which included all the patients who underwent randomization and had surgical resection of the primary tumor according to protocol.

Survival outcomes, including iDFS, DDFS, and OS, were estimated using the Kaplan–Meier method, with two-sided 95% CI derived from the log-log method.

ctDNA analyses were predefined as exploratory. ctDNA was evaluated both as a binary variable (detectable vs. undetectable) at baseline, C3D1, and the pre-surgical timepoint (data not shown), and as a dynamic categorical variable reflecting molecular response. The latter included the following categories: no ctDNA at baseline; ctDNA clearance (ctDNA detectable at baseline and undetectable at subsequent timepoints); and no ctDNA clearance (ctDNA detectable at baseline and remaining detectable at subsequent timepoints). Statistical comparisons were performed between detectable vs. undetectable ctDNA, and between no ctDNA clearance vs. ctDNA clearance groups. Association between baseline ctDNA status and clinicopathological variables, including disease stage and nodal status, were assessed using Fisher exact test. Binary outcomes, including pCR, were analyzed using logistic regression with Firth's penalized likelihood to reduce small-sample bias and account for potential separation, whereas time-to-event outcomes (iDFS, DDFS) were analyzed using Firth's penalized Cox proportional hazards regression unadjusted and adjusted models for clinically relevant covariates, including disease stage and nodal status. Hazard ratios were presented with corresponding 95% CI. All statistical tests were two-sided, and a significance level of $\alpha=0.05$ was applied. P values should be interpreted as exploratory, and no adjustment for multiple testing was performed.

Analyses were performed using R (version 4.3.2).

Declarations

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AUTHOR CONTRIBUTION STATEMENT

JC and AL-C formulate the initial concept of the study. JMPG, JC, and AL-C contributed to the final study design. MRB, AS, BB, SEDR, CR, CA, NMJ, VI, MC, NR made valuable contributions as investigators. FD, AP, PS, GG, FM, SC and SB were members of the trial Steering Committee. EMG, AM and MM administrated the study ensuring its successful execution. JAG, DAL and LM analyzed the data. JMPG, GA, GN, MG, JGM, LS, JRM, AAG, JC, and AL-C contributed to data interpretation. DD performed ctDNA assay experiments. JMPG wrote the first draft of the paper. DAL, JAG and AAG were responsible for the creation of the tables and figures in the study. All authors were involved in the revision and discussion of the paper. All authors reviewed and approved the final version of the manuscript for submission.

COMPETING INTEREST STATEMENT

JMPG declares honoraria for advisory board participation from Lilly, Roche, Eisai, Daiichi-Sankyo, AstraZeneca, Seattle Genetics, Merck Sharp&Dohme, and Gilead; personal support for attending meetings and/or travel, accommodations and expenses from Roche; being an employee at MEDSIR. JC declares honoraria from Roche, Novartis, Eisai, Pfizer, Lilly, Merck Sharp&Dohme, Daiichi Sankyo, Astrazeneca, Gilead, Steamline Therapeutics, Zuellig Pharma; cosulting/advisor from Roche, AstraZeneca, Seattle Genetics, Daiichi Sankyo, Lilly, Merck Sharp&Dohme, Leuko, Bioasis, Clovis Oncology, Boehringer Ingelheim, Ellipses, Hibercell, BioInvent, Gemoab, Gilead, Menarini, Zymeworks, Reveal Genomics, Scorpion Therapeutics, Expres2ion Biotechnologies, Jazz Pharmaceuticals, Abbvie, BridgeBio, Biontech, Biocon, Circle Pharma, Delcath Systems, Inc., Hexagon Bio, Bliss Biopharmaceutical, Relay Therapeutics; research funding to the institution from Roche, Ariad pharmaceuticals, AstraZeneca, Baxalta GMBH/Servier Affaires, Bayer healthcare, Eisai, F.Hoffman-La Roche, Guardant health, Merck Sharp&Dohme, Pfizer, Piqur Therapeutics,Iqvia, Queen Mary University of London; stock from MAJ3 Capital, Leuko (relative); travel, accommodation, expenses from Roche, Novartis, Eisai, Pfizer, Daiichi Sankyo, Astrazeneca, Gilead, Merck Sharp&Dhome, Steamline Therapeutics. Patents: Pharmaceutical Combinations of A Pi3k Inhibitor And A Microtubule Destabilizing Agent.Javier Cortés Castán, Alejandro Piris Giménez, Violeta Serra Elizalde. WO 2014/199294 A. ISSUED; Her2 as a predictor of response to dual HER2 blockade in the absence of cytotoxic therapy. Aleix Prat, Antonio Llombart, Javier Cortés.US 2019/ 0338368 A1. LICENSED. MRB declares having received speaker grants from Daiichi-Sankyo, AstraZeneca, Novartis, Lilly, Pfizer, Gilead and Roche; advisory board fees from Daiichi-Sankyo, AstraZeneca, Pfizer, Gilead and MSD; travel fund from Pfizer, Novartis, Roche, Daiichi-Sankyo and AstraZeneca. AS declares having received honoraria for speaker bureau from Daiichi-Sankyo, Novartis, AstraZeneca, Gilead; travel, accommodations and expenses from Eisai, Novartis, Pfizer, Gilead, Menarini; advisory role from AstraZeneca, Novartis, MSD and Gilead; member of GEICAM’s Steering Committee.

BB declares having received honoraria for advisory board participation from Novartis, Lilly, Menarini, and AstraZeneca; speaker's bureau from Genetec, Pfizer, Merck Sharp&Dohme, and Daiichi Sankyo; personal support for travel, accommodations and expenses from Gilead Sciences, and Pfizer. SEDR declares having received honoraria from COR2ED, Daiichi Sankyo/AstraZeneca, Jazz Pharmaceuticals, Novartis, Pfizer, Roche, and Seagen; consulting/advisory roles for Daiichi Sankyo/AstraZeneca, Jazz Pharmaceuticals, Pfizer, Pierre-Fabre, Reveal Genomics, and Roche; travel, accommodation, and expenses from Daiichi Sankyo, Pfizer, and Seagen; research funding to the institution as local principal investigator for studies sponsored by Byondis, Daiichi Sankyo/AstraZeneca, Jazz Pharmaceuticals, Lilly, MedSIR, Roche, SOLTI, Synthon, and Zymeworks. CR, CA, NMJ, FD, GG, MC, LS, DD declare no conflict of interest. NMJ, EMG, AM, AAG, JAG, DAL, JRM, LM and MM declare being an employee at MEDSIR. VI declares having received honoraria for consulting/advisory board participation from AstraZeneca and MSD Oncology and travel, accommodations and expenses form Pfizer, AstraZeneca, Novartis, Roche, Gilead Sciences. NR declares having received honoraria for speakers' Bureau from Novartis, Pfizer, Daiichi-AstraZeneca, Gilead and research funding from Pfizer. AP. reports advisory and consulting fees from AstraZeneca, Roche, Pfizer, Novartis, Daiichi Sankyo, Ona Therapeutics, and Peptomyc, lecture fees from AstraZeneca, Roche, Novartis, and Daiichi Sankyo, institutional financial interests from AstraZeneca, Novartis, Roche, and Daiichi Sankyo; stockholder and employee of Reveal Genomics; patents filed PCT/EP2016/080056, PCT/EP2022/086493, PCT/EP2023/060810, PCT/EP2024/068197 and EP23383369.PS declares having received personal honoraria for advisory board participation from AstraZeneca, Bicycle Therapeutics, Daiichi Sankyo, Gilead, Lilly, MSD, Novartis, Olema Oncology, Pfizer, Roche, and Summit Therapeutics; and personal honoraria as an invited speaker from AstraZeneca, Daiichi Sankyo, Gilead, Menarini-Stemline, MSD, and Roche and institutional research grants (financial interests) from AstraZeneca, Genentech, and Roche. FM declares having received personal honoraria for advisory board participation from AstraZeneca, BioNTech, Daiichi Sankyo, Eisai, Genomic Health, Gilead Sciences, Immunicom, Incyte, MSD, Myriad, Nerviano, Novartis, Novocure, Roche, and Seagen; and personal honoraria as an invited speaker from AstraZeneca, Daiichi Sankyo, GSK, Lilly, and Pfizer. Institutional honoraria as an invited speaker were received from AstraZeneca, Daiichi Sankyo, GSK, Seagen, and Stemline Menarini. In addition, has served as coordinating or local principal investigator for clinical studies sponsored by multiple organizations, including AGO Research GmbH, AstraZeneca, Boehringer-Ingelheim, Daiichi Sankyo, Eisai, German Breast Group, Gilead Sciences, GSK, Lilly, Menarini-Stemline, MSD, Novartis, Roche, Seagen, and Vaccibody; institutional funding with financial interest was received from Boehringer-Ingelheim, Daiichi Sankyo, and Menarini-Stemline. SDC declares having received an institutional grant from the Ministry of Health for the project PNRR-POC-2023-12378113; serving as an "ad hoc" medical monitor for Medica Scientia Innovation Research (MEDSIR), Barcelona (Spain); as well as support for attending meetings and/or travel from Daiichi Sankyo, and participation in an advisory board for Pfizer. SB declares having received honoraria for consulting/advisory board from Daiichi-Sankyo, AstraZeneca, Novartis, and Roche; speaker/conferences from Daiichi-Sankyo, AstraZeneca, Novartis, and Roche; and travel fees from Daiichi-Sankyo, AstraZeneca, Novartis, and Roche. GA declares having received honoraria from MEDSIR. GN declares having received honoraria for travel, accommodation and expenses from Eli Lilly, MSD and Novartis. MG

declares having received honoraria from Novartis, Gilead, Astra Zeneca, Pfizer; personal support for attending meetings and/or travel from Roche, Pfizer, Astra Zeneca, Gilead. Honoraria for advisory board participation from Gilead, Novartis, AstraZeneca, Pfizer. JGM declares having received honoraria for consulting or advisory role from MEDSIR; travel, accommodations and expenses from Novartis, Lilly, Roche, Daiichi-Sankyo Europe GmbH and being an employee at Trialing Health S.L. ALC declares having received honoraria for research support from Roche, Agendia, Lilly, Pfizer, Novartis, Merck Sharp&Dhome, Gilead Sciences, Daiichi Sankyo;
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Patents: Her2 as a predictor of response to dual HER2 blockade in the absence of cytotoxic therapy.Aleix Prat, Antonio Llombart, Javier Cortés.US 2019/ 0338368 A1. LICENSED;
Stock or other ownership from MAJ3 Capital & Initia-Research.

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Tables

Table 1. Time-to-Event Efficacy Endpoints

	Group A (standard treatment)	Group B (adaptive treatment)	Group B/PET responders with pCR (no CT)
Population who underwent surgery	n=63	n=267	n=86
Invasive disease-free survival events	2 (3.2%)	26 (9.7%)	6 (6.9%)
Ipsilateral invasive breast tumor recurrence	0	3 (1.1%)	2 (2.3%)
Regional invasive breast cancer recurrence	0	3 (1.1%)	0
Contralateral invasive breast cancer	0	1 (0.4%)	1 (1.2%)
Distant recurrence	2 (3.2%)	14 (5.2%)	1 (1.2%)
Second primary non-breast cancer	0	4 (1.5%)	2 (2.3%)
Death	0	1 (0.4%)	0
5-year invasive disease-free survival rate % (95% CI)	96.7% (92.2 to 100.0)	89.5% (85.7 to 93.4)	92.4% (86.7 to 98.5)
Distant disease-free survival events			
Distant recurrence	2 (3%)	18 (7%)	1 (1.2%)
Death	0	2 (1%)	0
5-year distant disease-free survival rate % (95% CI)	96.7% (92.2 to100.0)	92.7% (89.5 to 96.0)	98.7% (96.3 to 100.0)
Overall survival events			
Death	1 (1.4%)	5 (1.8%)	0
5-year overall survival rate % (95% CI)	98.4% (95.3 to 100.0)	98.1% (96.5 to 99.8)	100.0% (100.0 to 100.0)

Figures

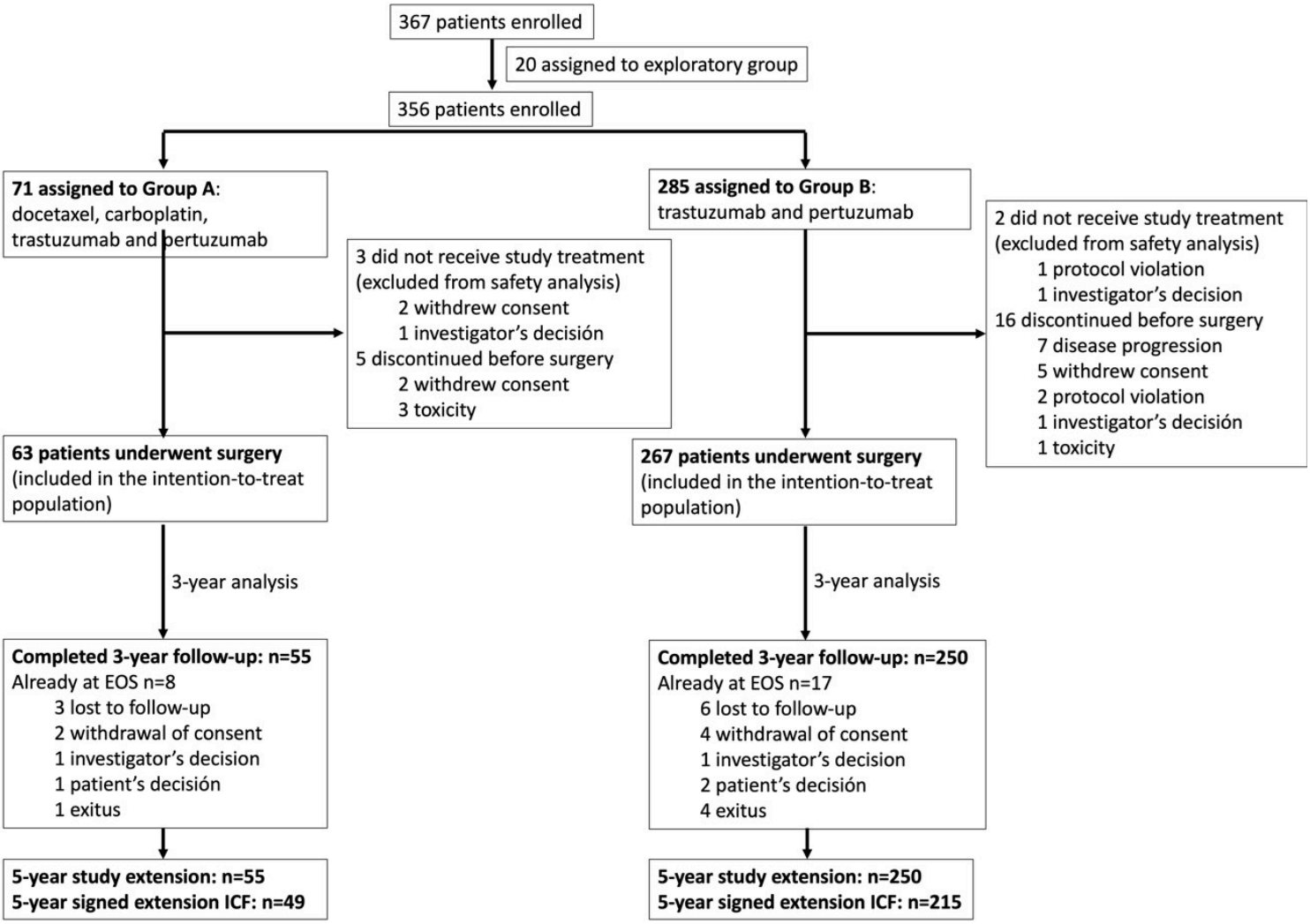


Figure 1

Trial profile

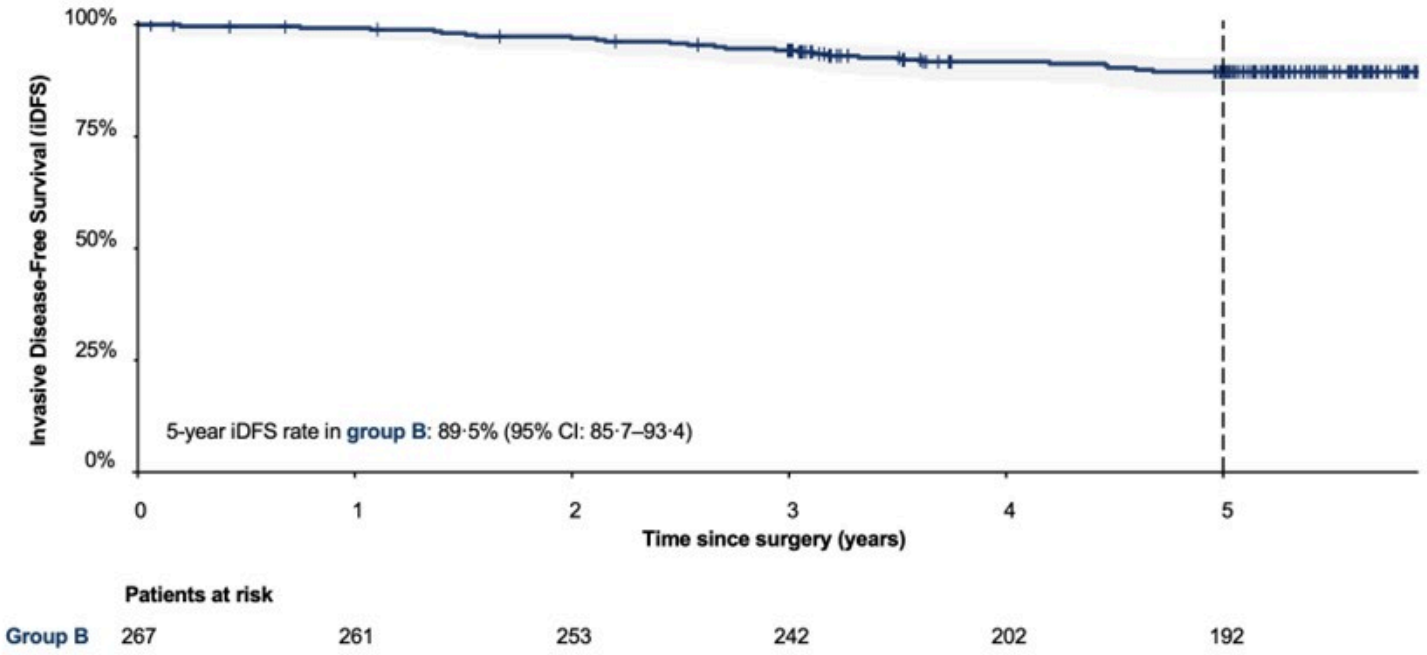


Figure 2

5-year invasive disease-free survival among patients included in group B.

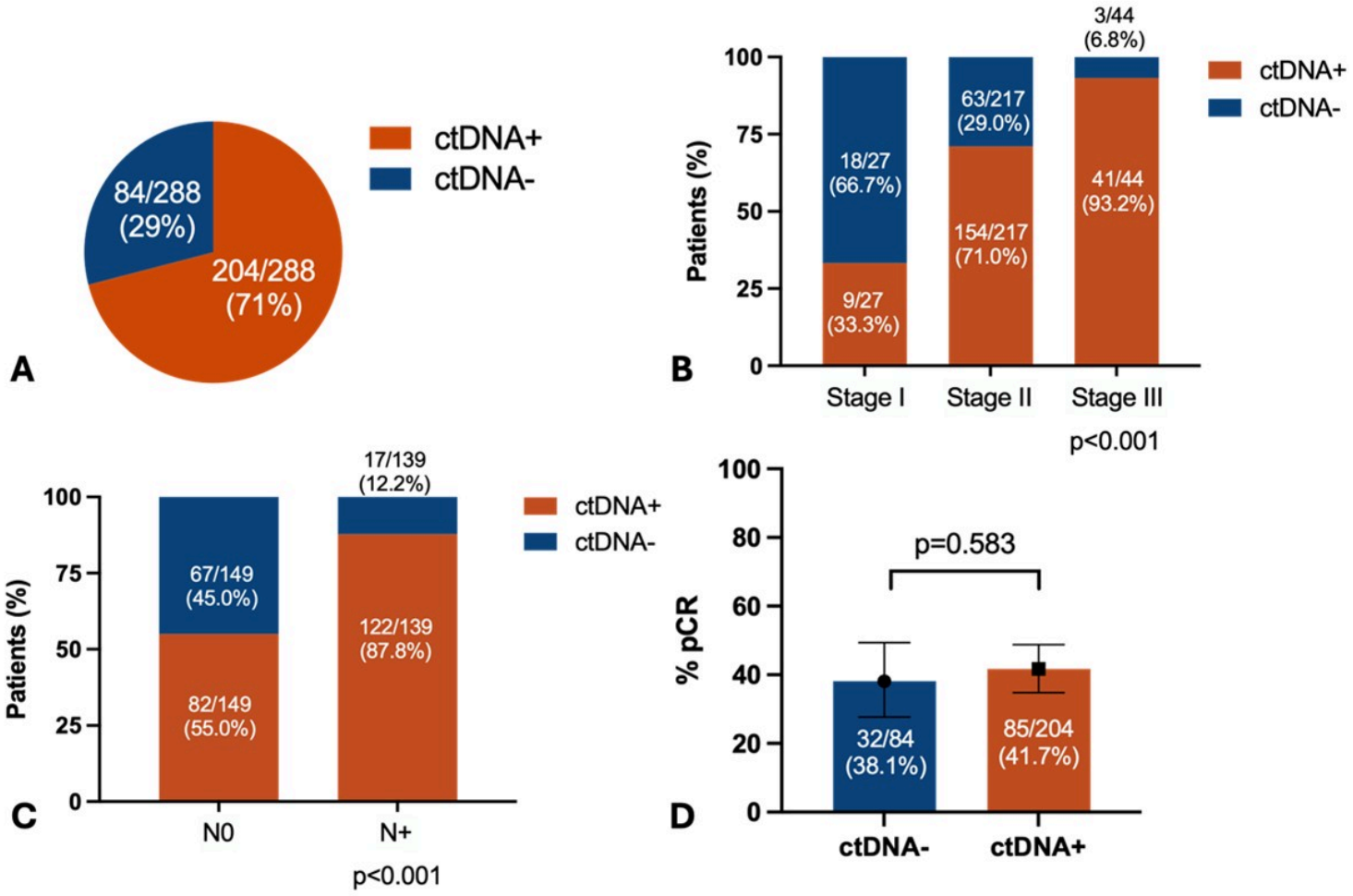


Figure 3

Association of baseline ctDNA detection with pCR and disease characteristics. N0: node-negative; N+: node-positive.

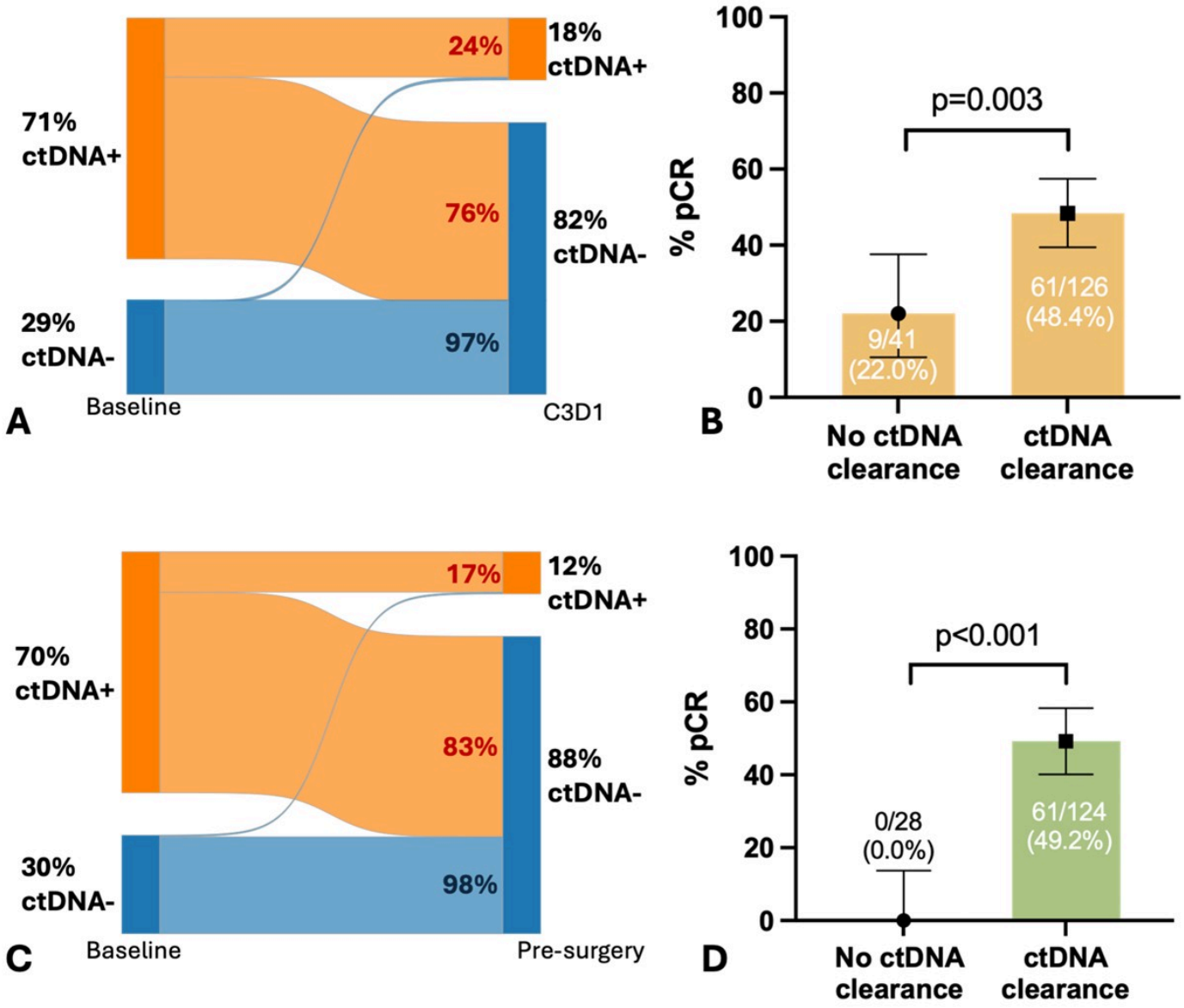


Figure 4

Association of ctDNA dynamics at C3D1 and pre-surgery with pCR.

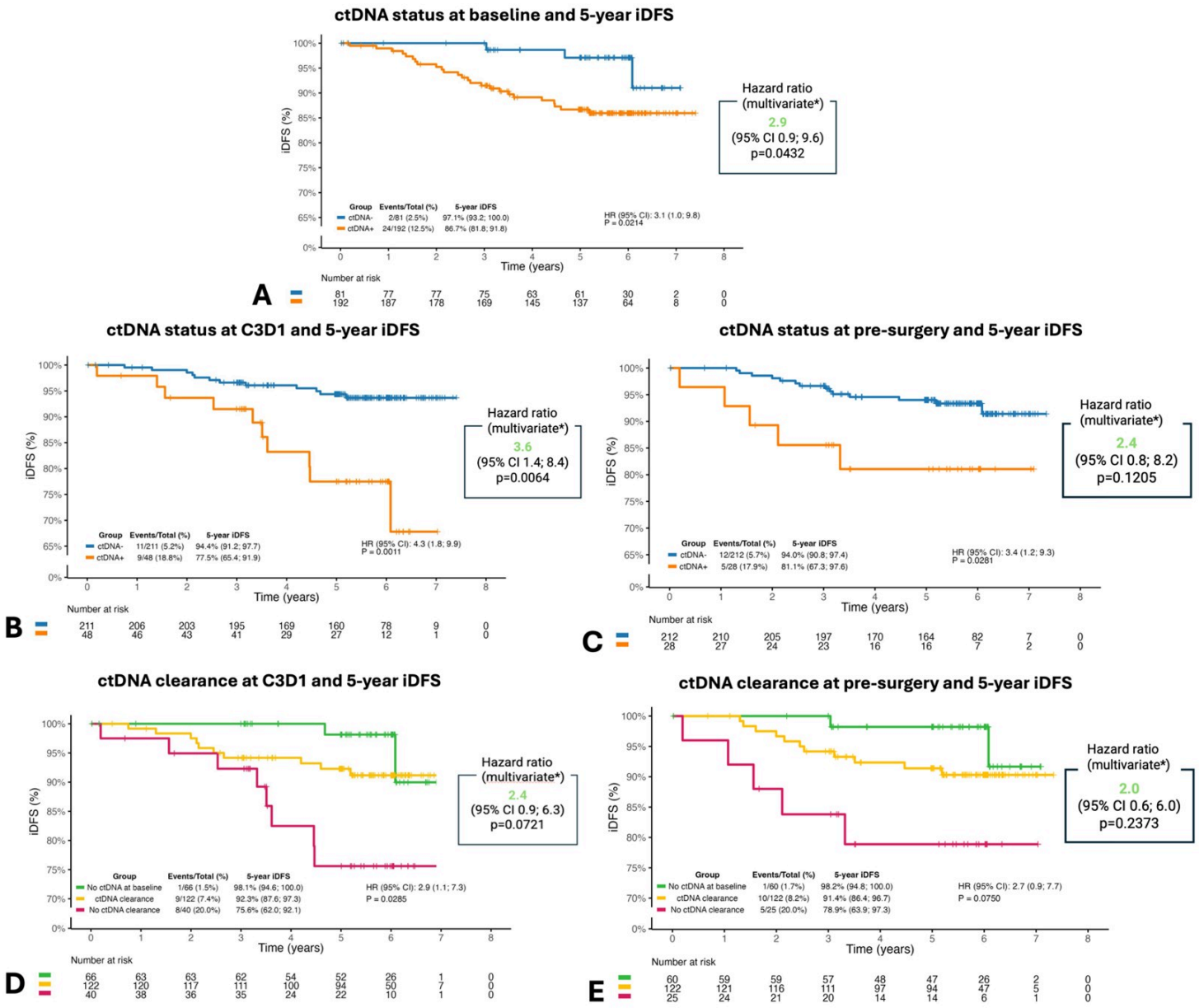


Figure 5

Association between ctDNA status and clearance at C3D1 and pre-surgery with 5-year iDFS.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PeXXrezGarciXXaJM.etal.SupplementaryMaterial.docx