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# Circulating Tumor DNA in HER2-Amplified Breast Cancer: A Translational Research Substudy of the NeoALTTO Phase III Trial

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## Abstract

**Purpose:** In the neoadjuvant treatment (NAT) setting, dual HER2-targeted therapy is associated with increased pathologic complete response (pCR) rates compared with each therapy alone. Biomarkers allowing to predict treatment response during NAT are needed. We aim to evaluate whether circulating tumor DNA (ctDNA) is associated with response to anti-HER2–targeted therapy.

**Experimental Design:** Plasma DNA collected before NAT, at week 2, and before surgery from patients enrolled in the NeoALTTO trial was assessed using digital PCR for *PIK3CA* and *TP53* mutation detection.

**Results:** A total of 69 of 455 (15.2%) patients had a *PIK3CA* and/or *TP53* mutation detected in the baseline tumor sample and evaluable ctDNA results from baseline samples. CtDNA was detected in 41%, 20%, and 5% patients before NAT, at week 2, and before surgery, respectively. ctDNA detection before NAT was significantly associated with older age and ER-negative status. ctDNA detection before NAT was associated with decreased odds of achieving pCR (OR = 0.15; 95% CI, 0.034–0.7; $P = 0.0089$), but not with event-free survival (EFS). Analyses for EFS were underpowered. Interestingly, the patients with HER2-enriched subtype tumors and undetectable ctDNA at baseline had the highest pCR rates. In contrast, patients with persistent ctDNA detection at baseline and week 2 had the lowest rate of pCR.

**Conclusions:** ctDNA detection before neoadjuvant anti-HER2 therapies is associated with decreased pCR rates. Interestingly, patients with HER2-enriched tumors and undetectable ctDNA at baseline had the highest pCR rates, therefore appearing as the best candidates for treatment deescalation strategies.

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## Introduction

In the neoadjuvant treatment (NAT) setting, dual HER2-targeted therapy is associated with an increased rate of pathologic complete response (pCR) in patients when compared with each therapy alone (1, 2). However, response to anti-HER2–targeted therapies is heterogeneous. Currently, there are limited strategies to predict and follow treatment responses over time.

Monitoring treatment response to neoadjuvant chemotherapy remains an area of unmet clinical need, as clinical and radiologic monitoring during treatment to predict pCR is frequently inaccurate. At present, there is no effective validated method to differentiate between responders and nonresponders or to monitor response in real-time during therapy. Early response to breast cancer NAT defined by a substantial reduction in tumor size appears to be a predictor of pCR when assessed by imaging (3), but remains an imperfect surrogate. Limited data have investigated whether patients with specific tumor molecular profiles benefit more than others from a given NAT regimen (4). It was recently shown that patients with HER2-enriched subtype tumors, as defined by the PAM50 classifier, are more likely to benefit from dual HER2 blockade therapies (5, 6). The identification of a novel molecular biomarker that can simultaneously capture information both on genomic profile and tumor burden, in patients prior to and during NAT is needed.

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## Translational Relevance

This translational research project aims to evaluate whether circulating tumor DNA (ctDNA) can be used as a biomarker to assess treatment response in patients with HER2-positive early breast cancer treated with neoadjuvant anti-HER2 therapy in the context of the NeoALTTO trial. We have shown that the detection of ctDNA before neoadjuvant anti-HER2–targeted therapies is associated with decreased probability of pCR. Moreover, patients with HER2-enriched subtype tumors and undetectable ctDNA at baseline had the highest pCR rates. These findings support the analysis of ctDNA as a novel biomarker for patient response to anti-HER2 treatment and to assist de-/escalating therapeutic strategies, therefore allowing to avoid overtreatment in patients at lowest risk, and more intensive approaches in patients showing suboptimal responses to therapy.

The use of cell-free circulating tumor DNA (ctDNA) as a minimally invasive biomarker is now being explored across many cancer types for a variety of clinical applications (7–10). In particular, monitoring of the disease and treatment response through the serial assessment of ctDNA appears as a very promising tool. However, only few studies have assessed the potential of ctDNA to monitor treatment response in the setting of NAT in breast cancer (10, 11). Here, we aimed to evaluate ctDNA as a tool to monitor treatment response and predict outcome to anti-HER2–targeted NAT in early breast cancer in the context of the NeoALTTO clinical trial (1). In particular, we aimed to assess the percentage of patients with detectable ctDNA in plasma samples collected before preoperative chemotherapy (baseline), after 2 weeks of treatment (week 2), and prior to surgery and correlate this with clinicopathologic features, gene expression signatures, PAM50 molecular subtypes, and clinical outcomes.

## Materials and Methods

### Patient population

The Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization [NeoALTTO, Breast International Group (BIG 1-06)] trial was a randomized, multicenter open-label phase III trial designed to assess the efficacy of dual inhibition of HER2 in patients with HER2-overexpressed and/or amplified primary breast cancer. Neoadjuvant treatment was given for 18 weeks with anti-HER2 therapies being given alone for the first 6 weeks, paclitaxel being added for the remaining 12 weeks. From January 2008 to May 2010, 455 patients were enrolled in the trial. pCR was defined as absence of tumor cells in the breast and lymph nodes (12), and event-free survival (EFS) was defined as the time from randomization to the first event (13). The median follow-up of the population was 6.64 years (range of 0.003–7.94 years). The patients who were meeting the following criteria were included in the present ctDNA analysis: (i) PIK3CA and/or TP53 mutations identified in the baseline tumor sample; (ii) available whole blood sample collected before NAT for ctDNA analysis. Detailed characteristics of the patients included in the NeoALTTO trial are provided in the study's main manuscript (1). The study was approved by the TransALTTO committee and was conducted in accordance with the Declaration of Helsinki. The trial was approved by relevant ethics committees and health authorities at all participating sites. Informed consent, including the participation to future biomarker research, was obtained from all participants.

### Sample collections

Whole blood samples from patients enrolled in the trial were collected before NAT (baseline), after 2 weeks of NAT (week 2), and before surgery. Blood samples were centrifuged within 2 hours of collection at 2,000–3,000 × *g* for 15 minutes at room temperature for plasma preparation, which was then stored at −80°C until DNA extraction. A second full speed centrifugation was performed after plasma thawing prior to circulating cell-free DNA (cfDNA) extraction. The extractions were performed from an average of 1.76 mL of plasma (range 0.15–3.25 mL) using the QIAamp Circulating Nucleic Acid Kit (Qiagen) according to the manufacturer's instructions with a DNA elution performed in 30 μL. A quality control step for cfDNA fragment size evaluation was performed using the Agilent Bioanalyzer according to the manufacturer's instructions.

### Droplet digital PCR analysis of *PIK3CA* and *TP53*-mutant ctDNA

The genomic and transcriptomic profiles of baseline tumor DNA and RNA from patients enrolled in the NeoALTTO trial were previously assessed using mass spectrometry–based genotyping (4), exome (14), and RNA (5) sequencing, respectively (Supplementary Table S7). Patients with identified *PIK3CA* and *TP53* mutations and available baseline plasma samples were selected for further analysis. The presence of ctDNA was assessed in plasma cfDNA samples using patient-specific droplet digital PCR (ddPCR) assays for the detection of the previously identified *PIK3CA* and *TP53* mutations, with a single mutation being selected for in each patient. When two mutations were identified in a single tumor sample, the mutation with the highest allelic fraction was selected if the 2 ddPCR assays were available and had comparable sensitivity.

Mutation-specific droplet digital PCR (ddPCR) assays consisted of either ready to use PrimePCR ddPCR Mutation Assays (Bio-Rad), or alternatively custom assays designed using the Bio-Rad Mutation Detection Assay online design tool. Detailed description of the ddPCR experiments and criteria for sample positivity are provided in the Supplementary Methods. Samples were double blinded to the experimenters.

### Statistical analysis

The Fisher exact test was used to evaluate differences in ctDNA detection according to the NeoALTTO stratification factors: clinical tumor size (T2 vs. T3/4), clinical nodal status (N0/1 vs. N2), hormone receptor (HR) status (positive vs. negative), and planned breast surgery (conservative vs. mastectomy). Kruskal–Wallis tests were used for associations between a continuous variable and a category. Associations between ctDNA detection and either pCR or EFS after adjustment for the NeoALTTO stratification factors were investigated using logistic and Cox proportional hazards regression, respectively. The *P* values were obtained using ANOVAs. ctDNA was assessed both as a categorical variable (positive vs. negative) and continuous variable (copies/mL plasma and variant allele fractions) in all analyses. Variant allele fractions (VAF) were normalized as log(VAF+0.01) while copies/mL plasma were normalized as log(copies+1). The HRs

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and ORs for all analyses involving continuous variables represent ratios over the median absolute deviation. RNAseq data for PAM50 classification, Genomic Grade Index (GGI), 70-gene, and STAT1 immune signatures as well as *ESR1* and *ERBB2* single gene expression were obtained as described previously (5). GGI is designed to predict the histologic grade, while the 70-gene signature is designed to predict survival in breast cancer. Both belong to a large class of proliferation-driven signatures (15). All analyses were performed using R (version 3.4). Tumor-infiltrating lymphocytes (TIL) data were obtained as described previously (16). The study complies with the REMARK guidelines (17).

## Results

### Detection of *PIK3CA* and *TP53*-mutant ctDNA in the NeoALTTO cohort

We first sought to evaluate the percentage of patients with detectable ctDNA in plasma samples collected before preoperative chemotherapy (baseline), after 2 weeks of treatment (week 2), and prior to surgery from patients enrolled in the NeoALTTO trial. A total of 139 of 455 (31%) patients had *PIK3CA* and/or *TP53* mutations identified in the primary breast tumor sample prior to the neoadjuvant treatment (4, 5, 14). Of these, 124 patients had an available baseline plasma sample and were included in the ctDNA detection analysis. Of note, 94 (75.8%) and 30 (24.2%) of the 124 patients had mutations in *PIK3CA* and *TP53* genes respectively. After selecting a single mutation per patient, patient-specific droplet digital PCR (ddPCR) assays were designed to detect *PIK3CA* and *TP53* mutations and accurately quantify mutant ctDNA in plasma. Five patients were included in an inter-laboratory pilot study that demonstrated excellent reproducibility between Jules Bordet and Peter McCallum institutes and were excluded from the present analysis. Of the remaining 119 patients, 69 of them had evaluable ctDNA results at baseline. Study workflow and reasons for patient exclusion are illustrated in Fig. 1. No differences in terms of clinical and pathologic characteristics were observed between the 124 cases and the whole NeoALTTO cohort (Supplementary Table S1). ctDNA was detected in 28 of 69 (41%), 13 of 65 (20%), and 3 of 60 (5%) patients before NAT (baseline), at week 2, and before surgery,

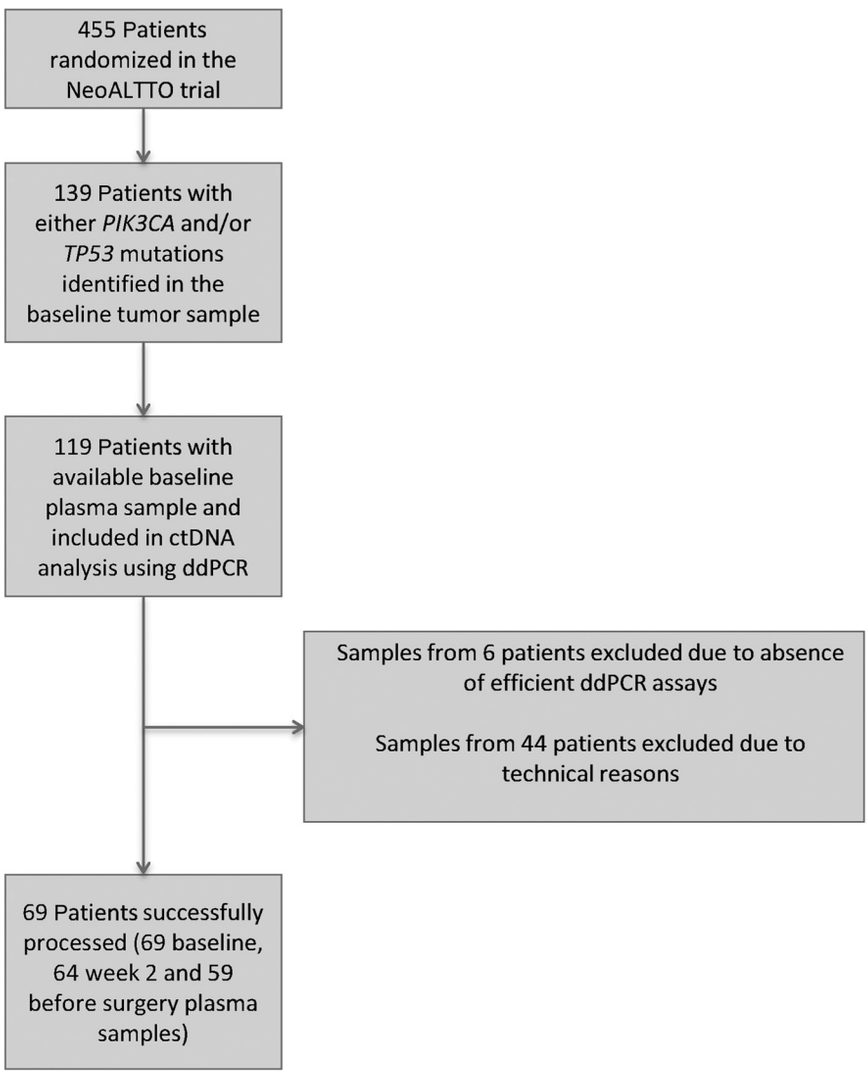


**Figure 1.**
NeoALTTO secondary analysis flow diagram of patients and samples.

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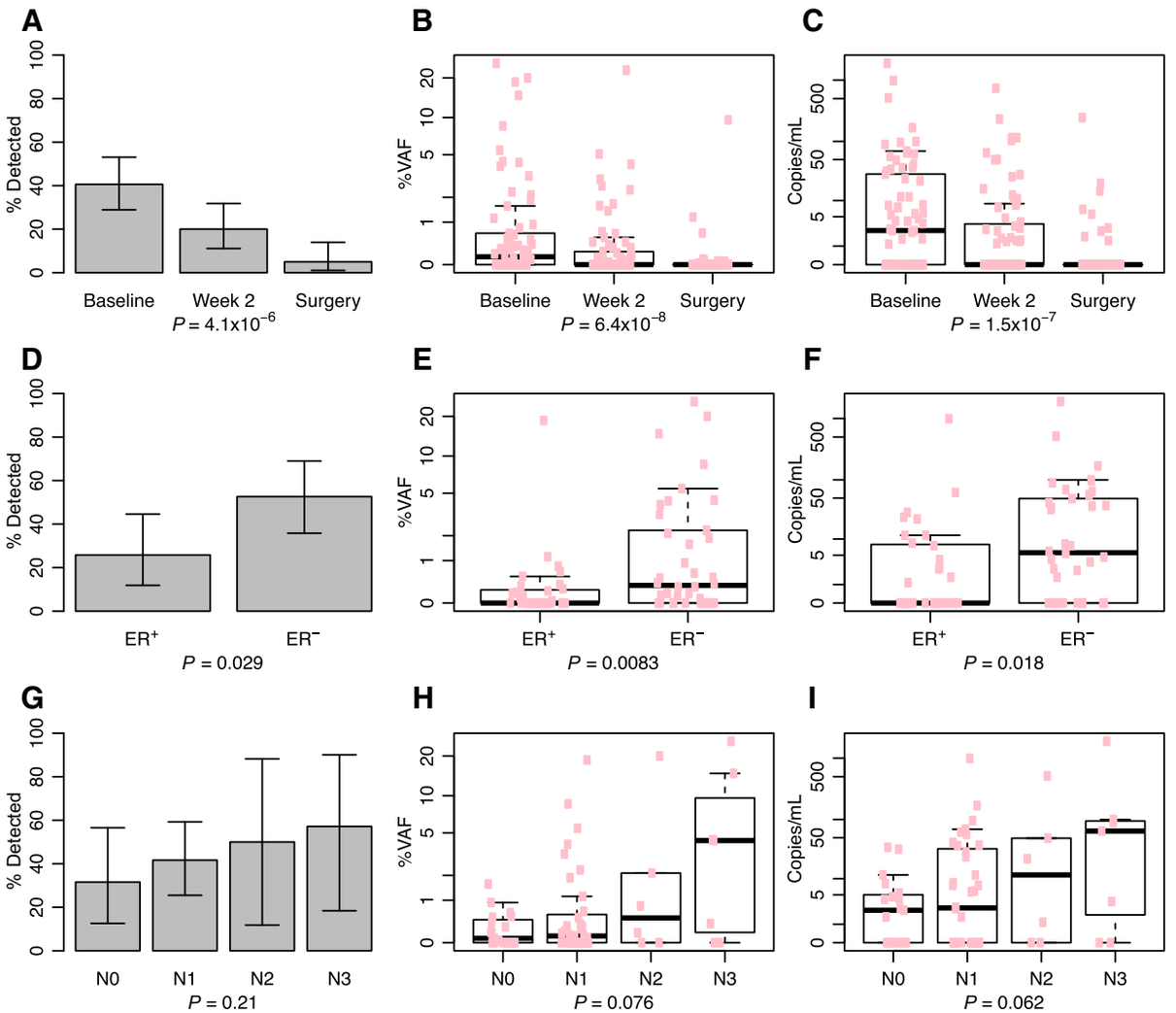


**Figure 2.**

Circulating tumor DNA (ctDNA) detection across the three timepoints, ctDNA detection considered as a binary variable in **A** and continuous variable in **B** and **C**. Association between ctDNA detection, considered as a binary or continuous variable, ER, and nodal status (**D–I**). Error bars are 95% confidence intervals on the proportions. VAF, variant allele fraction; ER, estrogen receptor; N, node stage.

respectively (Fig. 2A–C; Table 1). The levels of ctDNA varied between patients and across the timepoints evaluated; a median of 2.6 copies/mL plasma (range 0–1883.7 copies/mL) was observed at baseline (Table 1; Supplementary Table S2).

Of 22 patients with detectable ctDNA at baseline and available week 2 and presurgery plasma samples, ctDNA was still detectable in plasma samples from 8 patients at week 2 and detectable prior to surgery for only 2 patients (Supplementary Tables S2 and S3). Of note, ctDNA became detectable after 2 weeks of NAT for 4 patients who did not have detectable ctDNA at baseline (Supplementary Tables S2 and S3).

### Clinicopathologic features correlate with ctDNA baseline levels

We next investigated whether ctDNA detection or a change in ctDNA levels after NAT were associated with clinicopathologic features. The detection of ctDNA before NAT was significantly associated with ER status assessed by IHC ($P = 0.029$; Fig. 2D–F), but not with any other clinicopathologic features evaluated including clinical tumor size, nodal status, or type of planned breast surgery (Supplementary Table S4), with the exception of age, as we observed a higher detection rate in older patients ($P = 0.031$). An association between ctDNA detection and nodal status was also observed, although not significant (Kendal correlation tests; Fig. 2G–I). Changes in ctDNA levels were not significantly associated with specific clinicopathologic features (Supplementary Table S5).

### ctDNA detection and its association with specific gene expression signatures and PAM50 subtypes

Taking advantage of the availability of RNAseq data for 54 patients of this cohort, we further assessed whether ctDNA detection was different according to the PAM50 subtypes and whether it was associated with the expression of specific genes (including *ERBB2* and *ESR1*) and specific gene expression signatures

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**Table 1.** Frequency of ctDNA detection from the patients with evaluable ctDNA analysis

| Mutated gene | Timepoint | Samples analyzed (%) | Detectable ctDNA (%) | % VAF | Copies/mL plasma |
|---|---|---|---|---|---|
| *PIK3CA* and *TP53* | Baseline | 69 (100) | 28 (40.58) | 0.14% (0%–0.67%) | 2.6 (0–28.7) |
| *PIK3CA* | Baseline | 55 (100) | 24 (43.64) | 0.12% (0%–0.74%) | 2.8 (0–30.4) |
| *TP53* | Baseline | 14 (100) | 4 (28.57) | 0.18% (0%–0.39%) | 2.1 (0–5.7) |
| *PIK3CA* and *TP53* | Week 2 | 65 (100) | 13 (20) | 0% (0%–0.24%) | 0 (0–2.8) |
| *PIK3CA* | Week 2 | 51 (100) | 12 (23.52) | 0% (0%–0.28%) | 0 (0–3.4) |
| *TP53* | Week 2 | 14 (100) | 1 (7.14) | 0% (0%–0.16%) | 0 (0–1.3) |
| *PIK3CA* and *TP53* | Presurgery | 60 (100) | 3 (5) | 0% (0–0) | 0 (0–0) |
| *PIK3CA* | Presurgery | 46 (100) | 2 (4.34) | 0% (0–0) | 0 (0–0) |
| *TP53* | Presurgery | 14 (100) | 1 (7.14) | 0% (0–0) | 0 (0–0) |

capturing proliferation and immune signal (Fig. 3A–H). As shown in Fig. 3A, 44% of the tumors were classified as HER-2 enriched, whereas the remaining cases were classified as Normal-like (11%), Basal-like (13%), Luminal A (15%), and Luminal B (17%) according to the PAM50 classifier. ctDNA detection was significantly associated with the molecular subtypes, with the Basal-like and the Luminal-A subtypes having the highest (86%) and lowest (0%) ctDNA detection rates, respectively (Fig. 3B; Supplementary Table S6). Of interest, tumor samples with detectable ctDNA at baseline had higher levels of proliferation as witnessed by high GGI and GENE70 gene signatures (Fig. 3C and D) as well as low expression levels of *ESR1* gene (Fig. 3E). Detection of ctDNA at baseline was not significantly associated with ERBB2 expression level, TILs, or an immune signature (Fig. 3F–H).

### ctDNA detection at baseline is associated with decreased odds of pCR

We further evaluated whether ctDNA detection correlated with pCR (available for 67 of 69 patients) or EFS. Adjusting for NeoALTTO stratification factors (treatment arm, nodal status, tumor size, planned breast cancer surgery type, and HR status), ctDNA detection at baseline, considered as a binary variable, was associated with decreased odds of achieving pCR, (OR = 0.15; 95% CI, 0.034–0.7; $P = 0.0089$), but not with EFS (HR = 0.91; 95% CI, 0.24–3.5; $P = 0.89$; Fig. 4A; Supplementary Fig. S2). In contrast, neither ctDNA detection at week 2 nor prior to surgery was significantly associated with pCR nor EFS (Fig. 4B and C; Supplementary Fig. S2). Of note, our analyses were underpowered at these timepoints for these outcomes.

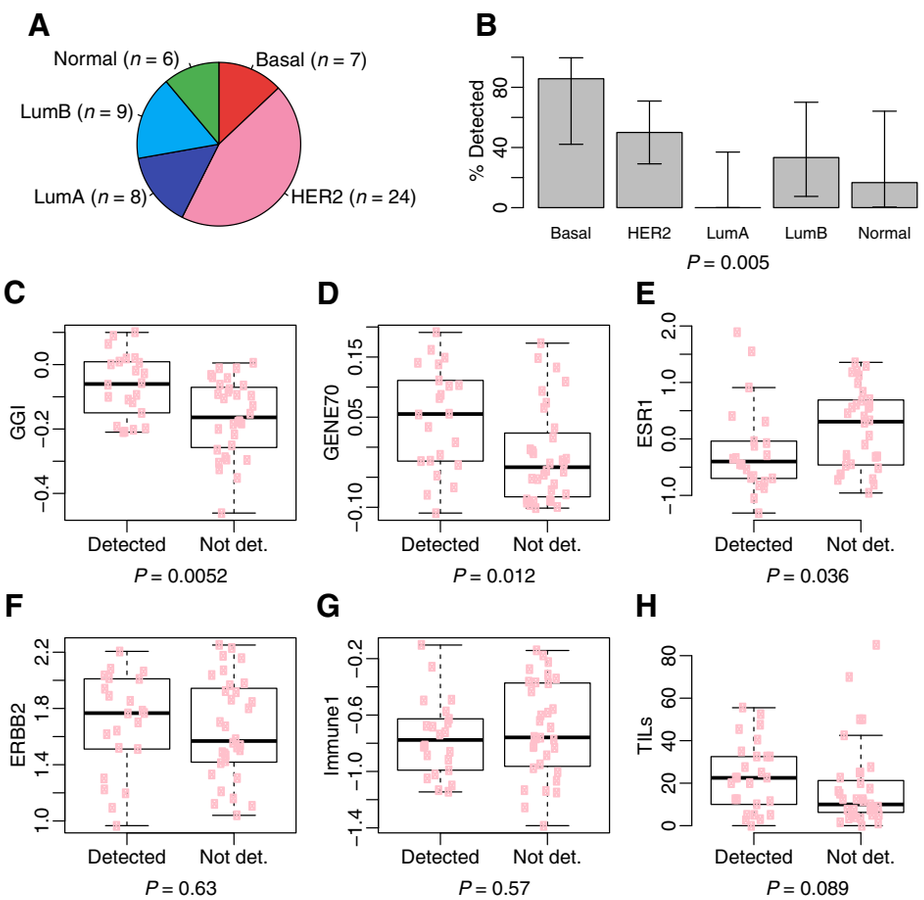


**Figure 3.**
Circulating tumor DNA (ctDNA) detection and its association with PAM50 subtypes, gene expression levels and gene signatures. Distribution of PAM50 subtypes in NeoALTTO substudy cohort including Luminal A and B (Lum A, LumB), Basal, HER2-enriched (HER2), and normal (**A**); ctDNA detection according to PAM50 subtypes (**B**); ctDNA detection and its association with proliferation gene signatures Genomic Grade Index (GGI) and GENE70 (**C** and **D**); ctDNA detection and its association with *ESR1* and *ERBB2* gene expression levels (**E** and **F**); ctDNA detection and its association with immune gene signature and TILs (**G** and **H**). Error bars are 95% confidence intervals on the proportions.

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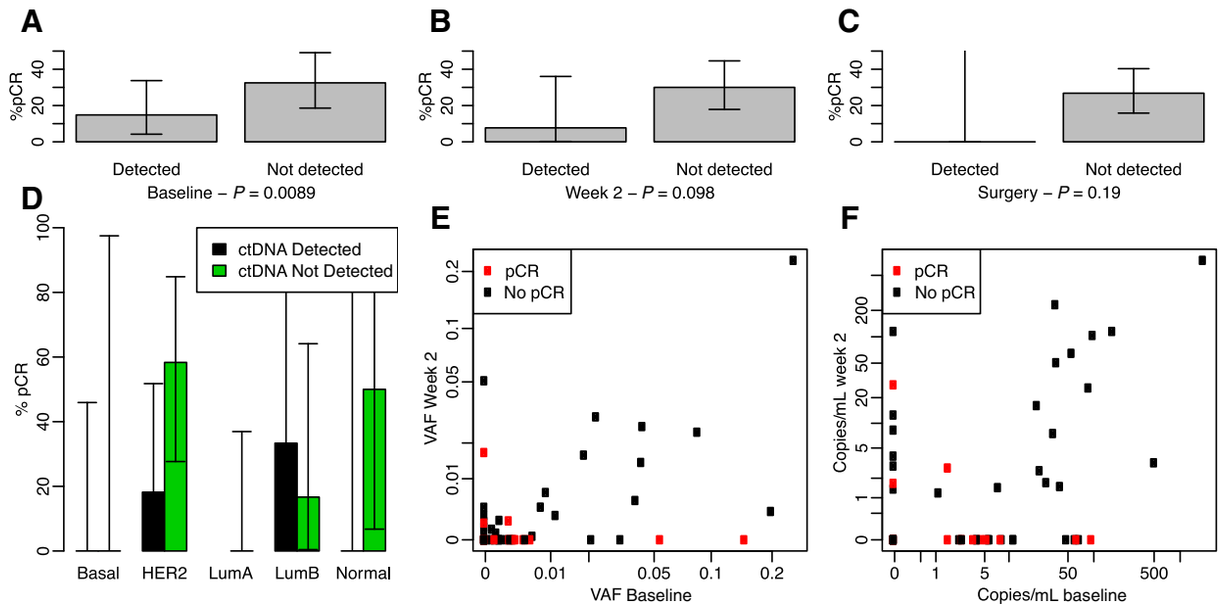


**Figure 4.**

Rates of achieved pCR according to ctDNA detection status at the 3 timepoints investigated (**A–C**), according to PAM50 subtypes at baseline (**D**) and considering changes in ctDNA detection between baseline and week 2 (**E** and **F**). VAF, variant allele fraction. Error bars are 95% confidence intervals on the proportions.

In addition, we explored whether ctDNA levels considered as a continuous variable were associated with pCR or EFS. These analyses revealed that ctDNA levels were less associated with pCR than the corresponding binary ctDNA analysis (Supplementary Fig. S2).

We next evaluated whether the association between the detection of ctDNA at baseline and the decreased odds of achieving pCR was different according to PAM50 intrinsic molecular subtypes. As previously shown (5, 6), the HER2-enriched subtype achieved higher pCR rates than other subtypes, although this result was not significant in the subcohort (OR = 3.9, $P = 0.11$). Moreover, among the patients with HER2-enriched subtype, those with undetectable ctDNA at baseline had the highest pCR rates in contrast with the other subtypes [HER2-enriched 7/12 (58%) vs. others 6/40 (15%), $P = 0.005$; Fig. 4D]. ctDNA detection remained associated with pCR after adjusting for PAM50 subtypes ($P = 0.002$).

### Changes in ctDNA levels after 2 weeks of NAT are neither associated with odds of pCR nor risk of recurrence

Finally, as we have previously demonstrated that dynamic changes in ctDNA levels can be informative to monitor treatment response in breast cancer (7), we investigated whether a change in ctDNA detection or levels after 2 weeks of NAT was associated with pCR or EFS, controlling for clinicopathologic parameters. These analyses showed that persistent ctDNA detection after 2 weeks of NAT was specifically associated with a lower pCR rate for patients in whom ctDNA was detected as baseline ($P = 7.3 \times 10^{-4}$, Supplementary Fig. S3A). This result, however, did not hold for EFS (Supplementary Fig. S3B). Early changes in ctDNA levels, analyzed as a continuous variable, were neither associated with pCR rate nor with the probability of recurrence (Supplementary Fig. S3A and S3B). However, patients with detectable ctDNA both at baseline and week 2 almost never achieved a pCR as shown in Fig. 4E and F ($P_{\text{interaction}} = 0.047$).

## Discussion

Neoadjuvant therapy has become standard practice in many patients with high-risk, early-stage breast cancer, where it has been shown to improve the rate of breast conserving surgery. Improved biomarkers are needed to monitor treatment response throughout therapy, guide the most effective delivery of NAT, and achieve improved outcomes. In the past years, ctDNA has appeared as a promising tool for the management of breast cancer offering a wide range of clinical applications ranging from disease monitoring to early detection of treatment response evaluation and relapse (8). The main objective of this study was to evaluate ctDNA as a means to monitor treatment response and predict outcome to anti-HER2–targeted NAT in early breast cancer in the context of the NeoALTTO clinical trial.

The presence of ctDNA was assessed through analysis of *PIK3CA* and *TP53* mutations, these genes being the two most frequently mutated genes in breast cancer (18). A total of 69 of 455 (15.2%) patients had *PIK3CA* and/or *TP53* mutations detected in the baseline tumor samples and evaluable ctDNA results from baseline. Our analyses showed that ctDNA was detected in 41%, 20%, and 5% patients before NAT, at week 2, and before surgery, respectively. The 41% detection rate prior to any treatment is similar to previous studies reporting a detection rate between 50% and 75% in patients with early-stage breast cancer, irrespective of the molecular subtypes (10, 11, 19, 20). Of note, our analysis was focused on the assessment of mutations in only 2 genes, namely *PIK3CA* and *TP53*.

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Interrogating additional mutations or copy number changes including *HER2* amplification may potentially have increased the rate of ctDNA detection. Nonetheless, our study represents the largest analysis to date of patients with early breast cancer receiving NAT for HER2-amplified breast cancer to report associations between ctDNA detection and pCR. We have shown that mutant ctDNA detected at baseline prior to NAT was associated with decreased odds of achieving pCR.

It has previously been shown within the NeoALTTO cohort, as well as other neoadjuvant trials of anti-HER2 therapy, that patients with tumors harboring *PIK3CA* mutations were less likely to achieve pCR (4, 14). The results support previous *in vitro* data highlighting the role of activating mutations in the PI3K signaling pathway, downstream of the HER2 receptor tyrosine kinase, in mediating resistance to HER2 therapy. These findings highlight a potential role of ctDNA testing to allow noninvasive analysis of genomic alterations, such as *PIK3CA* mutations, in patients receiving NAT. Clinical trials combining anti-HER2 therapy with PI3K inhibitors are currently underway to circumvent treatment resistance in this setting (NCT02705859). The ability to detect and follow mutation status through ctDNA analysis in patients with HER2-positive breast cancer may in the future allow selection of patients for consideration of combined therapy.

Our ctDNA analysis revealed no correlation between ctDNA detection during NAT and EFS, although our study was largely underpowered to detect such associations. Despite mutant ctDNA status not being predictive of EFS, we have shown that patients with detectable mutant ctDNA before preoperative treatment have lower probability of pCR.

To our knowledge, serial analyses of changes in ctDNA levels during NAT have only been previously studied in the context of patients with TNBC (10). In this study, ctDNA detection did not correlate with pCR at any timepoint; however, decreasing ctDNA levels were observed in patients responding to therapy, and ctDNA positivity after 1 cycle of NAT correlated with shorter disease-free and overall survival. In addition, patients with detectable ctDNA both at baseline and week 2 almost never achieved a pCR, although this observation was not significant probably due to small sample size. This finding suggests the potential value of serial ctDNA analysis during NAT to provide early information on therapy benefit. However, further validation is needed. This approach may allow risk stratification of patients to guide the intensity and duration of NAT regimens.

Recent evidence suggests that the HER2-enriched molecular subtype within the HER2-positive breast cancer is associated with increased rates of pCR following neoadjuvant dual HER2 blockade (5, 6). Although not significant due to limited sample size of the present NeoALTTO substudy, HER2-enriched subtypes achieved higher pCR rate. We have also shown that patients with HER2-enriched subtype tumors and undetectable ctDNA at baseline have the highest pCR rates suggesting that these patients may be the best candidates for future anthracycline-based chemotherapy de-escalation strategies. However, more patients are needed to further explore whether PAM50 subtyping and ctDNA detection both provide independent prognostic information for achieving pCR beyond classical clinicopathologic characteristics.

In conclusion, in the neoadjuvant setting, ctDNA has the potential to improve patient management by allowing genomic information and tumor burden to be monitored in parallel. Analysis of ctDNA can provide an early indication of treatment response/resistance and predict patients unlikely to achieve pCR. To facilitate optimal NAT regimens, individualized disease monitoring strategies using ctDNA could allow tailored treatment schedules to avoid overtreatment in patients at lowest risk, and more intensive approaches in patients showing suboptimal responses to therapy. Future clinical trials based on ctDNA risk stratification in the NAT setting will be needed to assess the clinical utility of this approach in the treatment of HER2-positive early-stage breast cancer.

## Disclosure of Potential Conflicts of Interest

E. de Azambuja reports receiving commercial research grants and other remuneration from Roche and is a consultant/advisory board member for Roche/GNE. S. Di Cosimo reports receiving speakers bureau honoraria from Novartis Pharma. S. K. Chia is a consultant/advisory board member for Hoffmann LaRoche. A. M. Wardley reports receiving speakers bureau honoraria from Roche, Pfizer, and Napp, and is a consultant/advisory board member for Roche, Lilly, Novartis, Pfizer, Daiichi Sankyo, Amgen, Athenex, Accord, and AstraZeneca. T. Ueno reports receiving speakers bureau honoraria from Novartis. W. Janni reports receiving commercial research grants from and is a consultant/advisory board member for Novartis. J. Huober reports receiving commercial research grants from Novartis, and reports receiving speakers bureau honoraria from and is a consultant/advisory board member for Roche and Novartis. J. Baselga is an employee of AstraZeneca, Foghorn, Varian Medical Systems, Bristol-Myers Squibb, Grail, Aura, and Infinity Pharmaceuticals; reports receiving other commercial research support from Roche; holds ownership interest (including patents) in PMV Pharma, Tango, Venthera, Seragon, Juno, and Northern Biologicals; and is a consultant/advisory board member for Eli Lilly and Novartis. S. Loi reports receiving commercial research grants from Novartis; reports receiving other commercial research support from Merck, Bristol-Myers Squibb, Roche Genentech, and PUMA Biotechnology; and is a consultant/advisory board member for Merck, Bristol-Myers Squibb, Novartis, Roche-Genentech, and Pfizer. M. Ignatiadis is a consultant/advisory board member for Celgene, Novartis, Roche, Tesaro, Seattle Genetics, and Pfizer. No potential conflicts of interest were disclosed by the other authors.

## Authors' Contributions

**Conception and design:** F. Rothé, D.D. Rosa, A.M. Wardley, W. Janni, S. Loi, C. Sotiriou, S.-J. Dawson, M. Ignatiadis

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