

# Neoadjuvant Chemotherapy for Breast Cancer: Past, Present, and Future

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**ABSTRACT:** Neoadjuvant chemotherapy (NAC) had been developed as a systematic approach before definitive surgery for the treatment of locally advanced or inoperable breast cancer such as inflammatory breast cancer in the past. In addition to its impact on surgery, the neoadjuvant setting has a benefit of providing the opportunity to monitor the individual drug response. Currently, the subject of NAC has expanded to include patients with early-stage, operable breast cancer because it is revealed that the achievement of a pathologic complete response (pCR) is associated with excellent long-term outcomes, especially in patients with aggressive phenotype breast cancer. In addition, this approach provides the unique opportunity to escalate adjuvant therapy in those with residual disease after NAC. Neoadjuvant chemotherapy in breast cancer is a rapidly evolving topic with tremendous interest in ongoing clinical trials. Here, we review the improvements and further challenges in the NAC setting in translational breast cancer research.

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## Introduction

Neoadjuvant chemotherapy (NAC) refers to systemic treatment before a definitive operation. In the past, NAC was reserved for patients with locally advanced or inoperable breast cancer with the primary purpose to reduce the tumor size (also known as downstaging) to allow breast-conservation surgery and possibly omit axillary dissection in patients who are opposed to an extensive operation. However, currently the role of NAC has expanded to include patients with early-stage, operable breast cancer. Thus, NAC allows improved cosmetic outcomes and reduces postoperative complications such as lymphedema.

As discussed below, clinical trials evaluating NAC versus adjuvant chemotherapy failed to show a difference in the long-term outcomes for early-stage and locally advanced breast cancer with either approach. Since different tumors and different individuals have varying degrees of chemo-sensitivity that influence long-term outcomes, NAC provides an *in vivo* validation model to test the efficacy of these treatments. The surrogate endpoint, response to chemotherapy is a strong predictive factor for the risk of recurrence, especially in triple-negative breast cancer (TNBC) and HER2-positive breast cancers.<sup>1–6</sup> The above benefits have resulted in wide-spread adoption of NAC. The use of neoadjuvant endocrine therapy in luminal A and B breast cancer subtypes is considered

investigational and not the focus of this review.<sup>7</sup> Herein, we review the results of NAC clinical trials and summarize the clinical features and molecular profiles of breast cancer for successful choice of NAC.

## NAC Clinical Trials

The first clinical trials with presurgical chemotherapy were reported by a group at the Milan Cancer Institute in the 1981.<sup>8</sup> The 2 combined approaches, chemotherapy (adriamycin plus vincristine) followed by radiotherapy or by mastectomy, was compared in a total of 132 women with locally advanced breast cancer. There was no significant difference in clinical outcome including local control, between the 2 treatment groups.

The National Surgical Adjuvant Breast and Bowel Project (NSABP) B-18 trial is the first trial in 1997 that compared neoadjuvant and adjuvant use of the same chemotherapy regimen.<sup>9</sup> A total of 1523 operable breast cancer patients were randomized to 4 cycles of doxorubicin and cyclophosphamide (AC) before (n = 747) or after surgery (n = 759). The hypothesis in this study was that the tumor response to chemotherapy before surgery correlates with outcomes and could serve as a surrogate marker for evaluating the effect of chemotherapy on undetected metastases. Tumor size reduced to less than half in 80% of the patients, but there was no significant difference in disease-free survival (DFS), distant disease-free survival, or overall survival (OS;  $P = .99, .70, \text{ and } .83$ , respectively). However,

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outcome was better in patients who achieved pathologic complete response (pCR) than in those who did not.<sup>10</sup>

European Organization for Research and Treatment of Cancer (EORTC) trial 10902 also compared neoadjuvant and adjuvant treatments of the same chemotherapy regimen. The outcomes between neoadjuvant and adjuvant administration of 4 cycles of fluorouracil, epirubicin, and cyclophosphamide (FEC) chemotherapy were compared among 698 operable breast cancer patients. There was no significant difference between the 2 groups in OS, DFS, or rate of loco-regional recurrence.<sup>11</sup>

To investigate the additional effect of combination chemotherapy, some randomized trials investigated the sequential use of taxane (T). In NSABP-B27 trial, patients were assigned to 3 groups, namely preoperative AC for 4 cycles followed by surgery, preoperative AC followed by T for 4 cycles and surgery, preoperative AC followed by surgery, and then postoperative T for 4 cycles. The addition of T to AC did not significantly influence DFS or OS; however, compared to AC followed by surgery group, the additional T to AC significantly increased the rate of pCR (26% versus 13%,  $P < .0001$ ). Patients who achieved a pCR continued to have significantly superior DFS and OS.<sup>2</sup>

The Gepar Duo study, using a dose-dense preoperative chemotherapy, was performed to compare the pCR rate between preoperative administration of dose-dense combination chemotherapy, doxorubicin 50 mg/m<sup>2</sup> plus docetaxel 75 mg/m<sup>2</sup> every 14 days for 4 cycles (ADOC) with filgrastim support, or doxorubicin 60 mg/m<sup>2</sup> plus cyclophosphamide 600 mg/m<sup>2</sup> every 21 days followed by docetaxel 100 mg/m<sup>2</sup> every 21 days for 4 cycles each (AC-DOC).<sup>12</sup> The pCR rate was significantly higher in AC-DOC group (14.3% versus 7.0%;  $P < .001$ ) along with the rate of breast-conserving surgery (63.4% versus 58.1%;  $P < .05$ ). Sequential AC-DOC was more effective at achieving pCR than dose-dense ADOC. The above studies suggest that pCR can serve as a surrogate marker for prediction of long-term outcome.

Although the above trials did not show any difference in long-term outcomes between NAC versus adjuvant chemotherapy, however, NAC enables identification of patients with chemotherapy-resistant disease, allowing the opportunity to tailor systemic therapy according to disease subtype. This post-surgical systemic therapy has been shown to improve long-term outcomes in residual disease.<sup>13,14</sup> At the same time, this approach spares the remaining population who achieve pCR the side effects of additional intensive interventions.

### Response to NAC Differ by Breast Cancer Subtypes

The US Food and Drug Administration established the Collaborative Trials in Neoadjuvant Breast Cancer (CTNeoBC) group, which analyzed 12 pooled neoadjuvant randomized controlled trials on pCR association with long-term outcome.<sup>15</sup> The group concluded that the association between pCR and long-term outcome was greatest in aggressive breast cancer subtypes. Pathologic complete response, defined as no pathological remnant tumor in the primary breast or lymph nodes except for in

situ disease (ypT0/is ypN0), was achieved the highest (50.3%) after trastuzumab, an anti-HER2 receptor monoclonal antibody in HER2-positive/hormone receptor (HR)-negative breast cancer. The pCR rates after NAC were also high in TNBC (33.6%), HER2-positive/HR-negative breast cancer treated without trastuzumab (30.2%), and grade 3 HR-positive/HER2-negative breast cancer (16.2%). In our recent retrospective clinical study, the pCR rates were significantly higher in HER2-positive/HR-negative breast cancer (52.9%) and TNBC (34.2%), compared to HR-positive breast cancer (14.7%).<sup>16</sup> In contrast, the association between pCR and long-term outcome was weakest for HR-positive subtypes, in which pCR (ypT0/is ypN0) rates were the lowest (7.5%). Lower pCR rates were also observed in luminal-A type tumors (6.4%), and higher rates in luminal B type tumors (11%–22%).<sup>6,13,17–19</sup>

Similar findings were observed in the I-SPY2 trial, where women with stage 2 or 3 breast cancer (low 70-gene assay score were excluded) were adaptively randomized to one of several different investigational agents with standard of care neoadjuvant therapy.<sup>20</sup> Rate of pCR was lowest for HR-positive, HER2-negative tumor (17.4%), and this increased approximately additively for HER2 positivity and HR negativity, up to 68% for HR-negative HER2-positive tumors. For patients receiving hormonal therapy, these rates were low, about 15% for HR-positive, HER2-negative tumors, 17% for HR-positive, HER2-positive tumors, and higher, about 21% in HR-negative, HER2-negative tumors, and 42% in HR-negative HER2-positive tumors. The 3-year event-free survival (EFS) was 95% for patients achieving pCR compared with 78% for non-pCR, with a hazard ratio of 0.19 (95% confidence interval [CI]: 0.12, 0.31). Similarly, 3-year distant recurrence-free survival (DRFS) was 95% for pCR versus 81% for without pCR, with hazard ratio 0.21 (95% CI: 0.13, 0.34).

### HER2 Directed Targeted Neoadjuvant Therapy for HER2-Positive Breast Cancer

In general, HER2 protein overexpression and/or HER2 gene amplification is found in about 25% to 30% of invasive breast cancers. It is well known that HER2-positive breast cancer has a more aggressive phenotype with higher relapse and mortality rates when untreated; however, the blockade of HER2 with specific agents has demonstrated significantly better prognosis. The first large study was conducted at the MD Anderson Cancer Center comparing the effect of NAC with or without HER2-targeted therapy with trastuzumab.<sup>21</sup> Forty-two patients with HER2-positive disease with operable breast cancer were randomly assigned to either paclitaxel followed by FEC for 4 cycles, or to the same chemotherapy regimen with trastuzumab in the NAC setting. Pathological complete response rates were 25% and 66.7% for chemotherapy alone, and chemotherapy plus trastuzumab, respectively ( $P = .02$ ). Despite the small sample size, the above study demonstrated that addition of trastuzumab to chemotherapy significantly improve pCR.

NeOAdjuvant Herceptin (NOAH) study was designed to assess the survival benefit from extended trastuzumab (in the neoadjuvant and adjuvant setting) in patients with HER2-positive locally advanced or inflammatory breast cancer.<sup>22</sup> Total of 235 patients were randomized to 2 groups; 1 year of treatment with trastuzumab (given as neoadjuvant and adjuvant treatment;  $n=117$ ) and no trastuzumab ( $n=118$ ). Neoadjuvant chemotherapy regimen consisted of doxorubicin, paclitaxel, cyclophosphamide, methotrexate, and fluorouracil. Disease-free survival was better in those with trastuzumab arm compared to those without trastuzumab (3-year DFS; 71% vs 56%, hazard ratio; 0.59 vs 0.013). Given this result, the combination of neoadjuvant and adjuvant trastuzumab to NAC should be considered for women with HER2-positive locally advanced or inflammatory breast cancer to improve long-term outcome.

The efficacy of lapatinib, a tyrosine kinase inhibitor (TKI), over trastuzumab, was evaluated in Gepar Quinto Setting III, where 620 patients with HER2-positive breast cancer were treated either with lapatinib or trastuzumab along with AT-based NAC.<sup>23</sup> The primary endpoint was pCR rate, which was analyzed in all patients who received at least one cycle of epirubicin and cyclophosphamide regimen (EC). Both HER2 inhibitors were given in combination with EC followed by taxane chemotherapy. Trastuzumab was more effective than lapatinib in achieving a higher pCR when given in combination with chemotherapy, 30.3% for trastuzumab group and 22.7% for lapatinib ( $P=.04$ ).

The efficacy of dual blockade of HER2 receptor by trastuzumab and lapatinib was investigated in NeoALTTO (Neoadjuvant Lapatinib and/or Trastuzumab Treatment Optimization) trial.<sup>24</sup> Among a total of 455 patients with HER2 overexpressing and/or amplified primary breast cancer, 154 patients received lapatinib alone, 149 trastuzumab alone, and 152 lapatinib plus trastuzumab. Anti-HER2 therapy alone was given for the first 6 weeks; weekly paclitaxel (80 mg/m<sup>2</sup>) was then added to the regimen for a further 12 weeks until surgery. Pathological complete response rate was significantly higher in the combination arm than in trastuzumab- or lapatinib-alone arms (51.3% versus 29.5% versus 24.7%,  $P=.0001$ ). There was no difference in pCR between the lapatinib and the trastuzumab alone arms ( $P=.34$ ). The total duration of the anti-HER2 therapy was 1 year. Hormone receptor-positive breast cancer had greater advantage of the dual HER2 blockade. These results show that the dual blockade of HER2 is better than one.

Addition of pertuzumab, another anti-HER2 receptor monoclonal antibody, was tested in NeoSphere trial, which showed the efficacy and safety of neoadjuvant pertuzumab and trastuzumab in women with HER2-positive breast cancer.<sup>25</sup> Patients were randomly assigned to trastuzumab plus DOC (group A), or pertuzumab plus trastuzumab plus DOC (group B), pertuzumab plus trastuzumab (group C), or pertuzumab plus DOC (group D). Patients in group B had significantly higher pCR of 45.8% compared with patients in group A with

29.0%. Pathological complete response in group C was 16.8% and group D was 24%. Hence, dual blockade of HER2 as well as addition of DOC result in significantly improved pCR compared with single agent with or without DOC. Patients who achieved pCR had longer 5-year progression-free survival (PFS) compared with patients who did not (85% versus 76%). This suggested that achievement of pCR could be an early indicator of long-term outcome in patients with operable HER2-positive breast cancer.

TRYPHAENA study is an open-label phase II study, where patients with operable, locally advanced, or inflammatory HER2-positive breast cancer were randomized to 3 groups; FEC plus trastuzumab plus pertuzumab followed by DOC plus trastuzumab plus pertuzumab (Arm A), FEC followed by T plus trastuzumab plus pertuzumab (Arm B), T plus carboplatin plus trastuzumab plus pertuzumab (Arm C).<sup>26</sup> Pathological complete response was 61.6% in Arm A, 57.3% in Arm B and 66.2% in Arm C patients. The authors also reported that dual HER-2 targeting resulted in low rates of symptomatic left ventricular end-systolic dimension. Three-year survival estimates for DFS were 87%, 88%, and 90% in groups A to C, respectively. Progression-free survival rates were 89%, 89%, and 87%. Hazard ratio of DFS was 0.27 compared between pCR and non-pCR. These above trials show that targeting HER2 in the NAC setting with dual blockade improves pCR rate, which translates into long-term survival benefit.

To test if pCR improvement in the neoadjuvant setting would translate into improved outcomes if the same treatment approach is used in the adjuvant setting, ALTTO trial<sup>27</sup> tested the hypothesis that dual anti-HER2 blockade would improve outcomes, given the positive findings in the sister NeoALTTO trial. A 16% reduction in the hazard of a DFS event was observed with lapatinib and trastuzumab, compared to trastuzumab alone. However, this effect was modest, not statistically significant, and also of little clinical significance in consideration of additional toxicity. This trial provides an important lesson that although achieving pCR is beneficial, it may not always translate into improved outcomes. Given these results, neoadjuvant trastuzumab with lapatinib has not been adopted into practice.

The role of NAC using HER2-targeted agents is even more highlighted, given the results of KATHERINE trial for resistant disease.<sup>13</sup> This study randomized patients with HER2-positive residual invasive breast cancer in breast or axilla after neoadjuvant treatment with NAC plus trastuzumab to receive either TDM-1 (a.k.a. trastuzumab emtansine; an antibody-drug conjugate of trastuzumab and cytotoxic agent DM1,  $N=743$ ) or continue with adjuvant trastuzumab alone ( $N=743$ ) in the adjuvant setting. There was a significant improvement in DFS in the TDM-1 group compared with trastuzumab alone group (hazard ratio 0.50, 95% CI: 0.39, 0.64,  $P<.001$ ). TDM-1 is now the standard of care for management of residual disease after NAC for HER2-positive breast cancer.

### NAC for TNBC and Subgroup Classification Using Gene-Expression Profiles

Triple-negative breast cancer is defined by the lack of expression of ER, PR, and HER2, seen in approximately 13% of all breast cancer patients. Triple-negative breast cancer patients respond significantly better to NAC compared with ER-positive subtype, most likely because they are more proliferative. Indeed, it has been reported that the patients with TNBC ( $n=255$ ) had significantly higher pCR after NAC compared with non-TNBC ( $n=863$ ; 22% versus 11%;  $P=.034$ ).<sup>17</sup> This also forms the rationale to test novel agents in the NAC setting along with the existing chemotherapy backbone such as I-SPY clinical trials. Although TNBC initially respond to NAC well, it is associated with an unfavorable prognosis due to the absence of targeted therapies, heterogeneity, and aggressive nature of the disease.

In Gepar Quinto trial, the addition of bevacizumab to neoadjuvant anthracycline- and taxane-chemotherapy improved pCR.<sup>18</sup> The results from prespecified subgroup analyses suggest that bevacizumab benefit was mainly in TNBC (odds ratio, 1.67); however, due to no survival benefit, bevacizumab is not currently used in combination with NAC in breast cancer.<sup>17,19,28</sup>

Similarly, addition of platinum such as carboplatin to NAC has been tested in TNBC. This is based on the notion that breast cancer with BRCA 1 mutation where homologous recombination-based DNA repair is defected, is sensitive to interstrand cross-linking agents such as platinum analogs, and 80% of them are TNBC. CALGB 40603 is a phase II trial that investigated the benefit of adding carboplatin, bevacizumab, or the combination to taxane/anthracycline-based NAC in TNBC.<sup>29</sup> Four-hundred forty three patients with stage II/III TNBC were randomized to receive bevacizumab, carboplatin, or both bevacizumab and carboplatin. The addition of carboplatin significantly improved pCR compared with control, from 46% to 60% ( $P=.0018$ ). Combination of both agents resulted in highest pCR of 67%. However, there was no absolute benefit in 3-year EFS. On the other hand, in the Gepar Sixto trial, 595 patients with TNBC received paclitaxel, doxorubicin, and bevacizumab with plus/minus carboplatin.<sup>30</sup> Addition of carboplatin resulted in significantly improved pCR (53% vs 37%,  $P=.005$ ), translating into an absolute benefit in 3-year EFS of 9.7% over the control (85.8% vs 76.1%). It is important to note that the chemotherapy backbone used in GeparSixto was non-standard, including only anthracycline and taxane, without cyclophosphamide; while the CALGB study included cyclophosphamide as well, which could account for some of the differences observed in outcomes. The BrightTNess trial<sup>31</sup> is a phase 3, multicenter randomized study with a 2:1:1 randomization to veliparib + carboplatin addition, carboplatin addition, or standard of care to determine if veliparib (PARP inhibitor) contributed to higher pCR in patients receiving veliparib plus carboplatin plus paclitaxel, followed by doxorubicin and cyclophosphamide, compared with patients receiving paclitaxel only

followed by doxorubicin and cyclophosphamide. The pCR in veliparib and carboplatin arm was 53%, carboplatin arm 58% and 31% in standard of care. Addition of veliparib to carboplatin did not increase pCR compared to carboplatin alone. However, it was observed that the carboplatin addition to paclitaxel reduced the delivered dose intensity of paclitaxel. Long-term outcome of BrightTNess trial is not yet available.

Due to the toxicity, addition of carboplatin compromised the completion of intended chemotherapy in CALGB study; however, in BrightTNess trial, a strategy was used to minimize missed chemotherapy doses by delaying treatment to allow time for recovery from toxicities, rather than omitting planned chemotherapy doses. This allowed for a higher dose delivery in BrightTNess trial compared to the CALGB study. Although platinum analogs are considered to be more effective in tumors with BRCA mutation, surprisingly in a post hoc analysis in GeparSixto, gBRCA mutation carriers had higher pCR and DFS regardless of whether carboplatin was used or not. In fact, it was only gBRCA wild-type patients who actually achieved a pCR and DFS benefit with carboplatin.<sup>32</sup> Similarly, the INFORM study<sup>33</sup> compared cisplatin versus doxorubicin-cyclophosphamide chemotherapy in stage I to III HER2-negative breast cancer, with pCR of 18% in cisplatin arm, versus 26% in doxorubicin-cyclophosphamide arm with risk ratio of 0.70 (95% CI: 0.35, 1.4). These data exemplifies that BRCA mutation carriers are more sensitive to DNA damaging agents and not specifically to platinum per se (both doxorubicin and cyclophosphamide are DNA-damaging agents). Given the inconsistency in long-term outcomes from GeparSixto and CALGB study, and potential for increased toxicities and delay in delivery of curative intent treatment, the inclusion of carboplatin as part of the neoadjuvant therapy for patients with TNBC remains controversial.

The role of NAC in TNBC is further exemplified by the additive benefit of capecitabine in the adjuvant setting. This is demonstrated by the CREATE-X trial where 900 patients with stage I to IIIB, HER2-negative breast cancer who had received NAC and did not achieve pCR, received either placebo or capecitabine as postoperative adjuvant therapy (in addition to hormonal therapy for HR-positive disease). Disease-free survival was longer in the capecitabine group than in the control group (74.1% vs 67.6%). Five-year OS was also higher in the capecitabine group (89.2% vs 83.6%,  $P=.01$ ). This study emphasized that patients with higher risk of recurrence could be identified post-NAC and could derive additional benefit from adjuvant chemotherapy.<sup>14</sup>

There has been an interest in the whole field of oncology on use of checkpoint inhibitors as immunotherapy to harness immune system, and breast cancer was no exception.<sup>34</sup> KEYNOTE-522 is a clinical trial which randomized TNBC patients with previously untreated stage II or III disease to receive NAC with pembrolizumab (anti-PD-1 antibody at a dose of 200 mg) plus doxorubicin and cyclophosphamide (AC),

paclitaxel and carboplatin (784 patients; the pembrolizumab-chemotherapy group) followed by adjuvant pembrolizumab or placebo plus AC, paclitaxel and carboplatin (390 patients; the placebo-chemotherapy group).<sup>35</sup> Higher rate of pCR was observed with the addition of pembrolizumab (64.8% vs 51.2%,  $P < .001$ ). Similarly, another phase 3 randomized study IMpassion 031<sup>36</sup> randomized stage II and III TNBC to atezolizumab + nab-paclitaxel followed by atezolizumab and AC, or placebo + nab-paclitaxel followed by AC. Pathological complete response was observed in 57.6% in the atezolizumab arm, versus 41.1% in the placebo arm ( $P = .0044$ ). These results are promising, but EFS is immature at this time. Hence, currently ACT regimen remains the standard of care as NAC for TNBC.

### Pathological, Clinical, and Molecular Profiles as Biomarkers to Predict pCR and Tumor Recurrence After NAC

Current standard of care for breast cancer is based on subtypes, which is determined by the expression of ER, PR, and HER2 as they have been validated as prognostic and predictive biomarkers for therapy with higher pCR in TNBC and HER2-positive subtypes. Since pCR after NAC is associated with an improved DFS, it is critical to identify effective biomarkers for predicting pCR and designing clinical trials to target these novel biomarkers to achieve higher pCR in addition to subtypes. Indeed, it was observed that not all TNBC achieve pCR and some HR-positive/HER2-negative patients respond to NAC. Potential biomarkers that can predict therapeutic response to NAC not yet utilized in clinical practice but hold significant promise to alter management include Ki67 and tumor-infiltrating lymphocytes (TILs)<sup>37</sup>. In a study by Denkert et al,<sup>38</sup> Ki67 was significantly linked to prognosis in the complete cohort and in HR-positive, but not in TNBC.<sup>39</sup> Tumor-infiltrating lymphocytes are a mixture of proinflammatory immune cells such as cytotoxic CD8<sup>+</sup> T-cells, natural killer, dendritic, and T-helper cells, and those with immune suppressor action including B-cells and regulatory CD4<sup>+</sup> T-cells that are found in both the tumor and the surrounding microenvironment of breast cancer.<sup>40,41</sup> Tumor-infiltrating lymphocytes may have predictive value for NAC response and favorable long-term prognosis. Mao et al<sup>42</sup> performed meta-analysis to establish pooled estimates for pCR based on the presence of TILs in breast cancer from 13 studies. The detection of higher level of TILs before receiving treatment associated with higher pCR (OR = 3.93, 95% CI: 3.26, 4.73). By clinical subtype, TILs predicted higher pCR in TNBC (OR = 2.49, 95% CI: 1.61, 3.83), HER2-positive breast cancer (OR = 5.05, 95% CI: 2.86, 8.92), but not in HR-positive breast cancer (OR = 6.21, 95% CI: 0.86, 45.15). Programmed death ligand 1 is another exciting biomarker that can predict improvement in outcomes especially with the addition of checkpoint inhibitors in TNBC as observed in the clinical trial Impassion 130 with combination of atezolizumab and nab-paclitaxel in the metastatic TNBC setting.<sup>43</sup> However, in

the neoadjuvant setting in KEYNOTE-522<sup>35</sup> and IMpassion 031,<sup>36</sup> pCR improvements were observed irrespective of tumor PD-L1 status, which could be due to use of different drugs, or combination pathways, disease stage (early vs late), PD-L1 assays, or all of these factors.

It is known that TNBC responds heterogeneously to NAC, suggesting that there may be subsubtypes of TNBC. Six subgroups of TNBC were classified by gene expression: 2 basal-like (BL1 and BL2), an immuno-modulatory (IM), a mesenchymal (M), a mesenchymal stem-like (MSL), and a luminal androgen receptor (LAR) subtype.<sup>44</sup> According to the Prediction Analysis of Microarray (PAM50) classification, approximately 80% of clinical TNBC are classified as basal-like (BL1, BL2, IM, and M subtypes).<sup>45</sup> The pCR rate was the highest in BL1 (52%), and the lowest in BL2 (0%) and LAR (AR-positive TNBC, 10%) subgroups. However, despite the low pCR rate, DFS (85.7% vs 65.5%,  $P = .05$ ) and OS (95.2% vs 76.2%,  $P = .03$ ) were significantly better in patients with the LAR subtype.<sup>46-49</sup> Yu et al<sup>50</sup> found that androgen receptor (AR)-positive TNBC tumor has a high level of expression of “luminal-like” genes; therefore, it was associated with favorable prognosis compared to AR-negative TNBC.<sup>51</sup> Our group reported that AR-positive ER-positive breast cancer demonstrated significantly worse response to NAC but associated with better survival,<sup>52</sup> which implicate that AR may play a role in NAC response as well as survival regardless of subtypes. Overall, these data suggest that intrinsic molecular profiling provides clinically relevant information beyond current pathology-based classification and can serve as an independent predictor of pCR in patients who receive current standard chemotherapy regimens.

The development of high-throughput genomic technologies (e.g. microarrays and next generation sequencing) has enabled even more specific personalized cancer therapy.<sup>38,50,53</sup> I-SPY 2, a multicenter open-label phase II trial is designed to focus on the prediction of response to novel drugs in NAC and identification of biomarkers for definitive subsequent studies.<sup>54</sup> Several targets have been explored based on tumor biomarkers in this trial. Patients are categorized into 8 biomarker subtypes based on: HR and HER2 status, and risk level as assessed with the 70-gene assay by MammaPrint, Agendia (high-risk category 1 or 2). Neratinib and lapatinib, which are under clinical investigation for HER2-positive breast cancer, were also tested in this trial. Patients were assigned to receive traditional chemotherapy with either neratinib or trastuzumab. Pathological complete response rate was higher in neratinib group compared to trastuzumab group (56% vs 33%). PARP inhibitors, namely olaparib, veliparib, rucaparib, niraparib, and talazoparib are also under clinical investigation for BRCA-associated TNBC. In one of the I-SPY clinical trials, patients with TNBC were randomly assigned to receive paclitaxel with/without combination of veliparib with carboplatin (VC) followed by AC for 4 cycles. Pathological complete response rate was higher in VC group compared to the

control group (51% vs 26%).<sup>55-57</sup> All these aforementioned treatments remain under investigation, with current approval for NAC (ACT) in TNBC and NAC with pertuzumab and trastuzumab for Her2-positive breast cancer.

Five markers of DNA repair deficiency (BRCA1/2 germline mutation, PARPi-7, *BRCA1*ness, CIN70 expression signatures and PARP1 protein) and 70-gene ultra-high-risk biomarker signature (MP1/2) were proposed as mechanism-of-action-based biomarkers and performed as a prespecified analysis. BRCA-like is defined as the dysfunction of set BRCA gene or BRCA protein, caused by gene mutation, methylation in promotor or deletion, resulting in DNA repair deficiency. In particular, *BRCAness* is defined as a dysfunction of BRCA protein without gene abnormality. Gene-expression data from 116 HER2-negative breast cancer patients were compared between VC arm (n=72) and control arm (n=44) in the I-SPY 2 trial. It was reported that BRCA1/2 germline mutation, PARPi-7 and *BRCA1*ness were significantly associated with VC response ( $P=.03$ ), whereas neither CIN70 nor PARP1 protein specifically predicted VC response. This is explained by the lack of DNA repair mechanisms in BRCA-mutant and BRCA-like tumors which results in increased susceptibility to chemotherapy that cause DNA strand breaks.

In the future, molecular biology assessment might help to identify patients who can receive this excellent benefit from NAC. We have shown that predictive biomarkers like cell checkpoint molecules G2M and E2F pathway in are able to identify aggressive ER-positive tumors that metastasize followed by a poor prognosis, and correlate with higher response to NAC.<sup>58,59</sup> In addition, we also showed that a 4 gene score comprised of *DOK4*, *HCCS*, *PGF*, and *SHCBP1* was associated with more aggressive tumors and predicted for higher response to NAC.<sup>60</sup> Therefore, continued development of biomarkers in these arenas is essential to spare patients unnecessary toxicities from NAC, especially when there is no benefit of this approach.<sup>51,61,62</sup> Finally, despite the excellent prognosis associated with pCR, few cases of tumor recurrence have been still observed. We showed that HER2-positive status and advanced clinical stage defined as large tumor size and/or presence of lymph node metastasis before NAC were identified as significant predictors of tumor recurrence (9.8% for Her2 positive; cT3/T4 34.7%; cN1N3 11.2%).<sup>16</sup>

### Current Challenges in NAC

There are several challenging issues that need to be considered while recommending NAC to the patients. First issue is that the improvement in pCR is often higher than the improvement noted in OS. Despite this fact, many drugs developed in NAC setting are transitioned to the adjuvant setting, especially when NAC resulted in successful improvement in pCR. A glaring examples of this are no survival benefit noted with lapatinib (ALTT0 trial<sup>27</sup>), bevacizumab (BEATRICE<sup>63</sup>), or pertuzumab (APHINITY<sup>64</sup>). These examples warn us that benefits of novel agents in the NAC setting may not always

translate into the adjuvant setting. Conversely, discarding an unsuccessful agent in NAC setting based on low pCR and not testing it in the adjuvant setting may be erroneous. Neoadjuvant trastuzumab has less benefit in HR-positive than negative tumors; however, HR-positivity does not seem to influence its efficacy in the adjuvant setting.<sup>65,66</sup> Another issue with NAC is that it prolongs the period while the patient harbors the tumor. If the best pCR rate with NAC is around 40%, this means that the patient continues to harbor the tumor with ineffective drug in 60% of the cases. This may lead to the emergence of chemotherapy-resistant clones<sup>67</sup> or allow time for cancer to advance and metastasize. Finally, the thought of living with the cancer may be emotionally challenging to some patients, especially in cases where the tumor does not show immediate shrinkage. In addition, NAC can make the tumor margins softer and result in formation of fibrous tissue of uncertain significance (tumor beds), which can make subsequent surgical removal of the tumor technically challenging to secure adequate margins. The tumor margins are known to be exceedingly difficult to delineate, whether by palpation, ultrasonography, mammography, magnetic resonance imaging (MRI), or even histopathology after NAC. Therefore, it is of utmost importance that prior to initiating NAC, these cases should be evaluated with a multidisciplinary approach to ensure that pre-NAC, markers are placed accurately in both the tumor and positive lymph nodes, for better identification at the time of surgery.

### Future Perspective

NAC has the remarkable potential to convert an inoperable breast cancer into an operable one and allow a more conservative surgery with less morbidity and mortality. At present, our clinical practice focuses on administration of neoadjuvant therapy based on pathologically defined intrinsic subtypes of breast cancer (Luminal A, Luminal B, HER2-enriched, TNBC) with chemotherapy and HER2-targeted therapy. The development of molecular, genetic, and clinical biomarkers such as Ki-67, TILs, gene-expression profiling has had a considerable impact on our understanding of breast cancer biology, especially in HER2-positive and TNBC. Understanding the molecular, genetic, and clinical profiles and using validated prognostic and predictive tools will assist us in the decision-making process for more effective approaches toward further advancement of precision medicine. At the same time, we also should take a pause and think back whether we are really benefiting from NAC or discarding a promising novel agent for adjuvant therapy simply because it was unsuccessful in the neoadjuvant setting. It is important to remember that although both neoadjuvant and adjuvant treatments are meant to work on dormant cells or undetectable metastatic tumor, neoadjuvant treatment is able to provide us the additional information about tumor chemosensitivity. Future biomarker-based prospective clinical trials should help us answer these questions to enable practicing biomarker-based personalized medicine.

## Author Contributions

Conceptualization, K.T. and T.I.; investigation, M.A. and S.G.; writing—original draft preparation, M.A. and S.G.; writing—review and editing, K.T. and T.I.; supervision, K.T. and T.I.

## Informed Consent

This study examined human data that had been generated in the past by other studies. Informed consent was therefore not obtained in this study.

## Research Involving Human Participants and/or Animals

This article does not contain any studies with human participants or animals performed by any of the authors.

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