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Post-neoadjuvant treatment and the management of residual disease in breast cancer: state of the art and perspectives

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Abstract: Achieving a pathologic complete response after neoadjuvant treatment is associated with improved prognosis in breast cancer. The CREATE-X trial demonstrated a significant survival improvement with capecitabine in patients with residual invasive disease after neoadjuvant chemotherapy, and the KATHERINE trial showed a significant benefit of trastuzumab-emtansine (TDM1) in human epidermal growth factor receptor 2 (HER2)-positive patients who did not achieve a pathologic complete response after neoadjuvant treatment, creating interesting alternatives of post-neoadjuvant treatments for high-risk patients. New agents are arising as therapeutic options for metastatic breast cancer such as the cyclin-dependent kinase inhibitors and the immune-checkpoint inhibitors, but none has been incorporated into the post-neoadjuvant setting so far. Evolving techniques such as next-generation sequencing and gene expression profiles have improved our knowledge regarding the biology of residual disease, and also on the mechanisms involved in treatment resistance. The present manuscript reviews the current available strategies, the ongoing trials, the potential biomarker-guided approaches and the perspectives for the post-neoadjuvant treatment and the management of residual disease after neoadjuvant treatment in breast cancer.

Keywords: breast cancer, chemotherapy, pathologic complete response, post-neoadjuvant, residual disease

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Introduction

Neoadjuvant treatment (NAT) consists in the administration of upfront systemic treatments before surgery, a modality initially conceived for patients with locally advanced or inoperable disease in order to obtain tumoral shrinkage and thereby increase the chances of surgical resection.[1] The use of NAT has increased in recent years due to its potential advantages, including the *in vivo* assessment of tumor response, the increased rates of conservative surgical procedures, and the possibility of starting an early treatment for micrometastatic disease.[2] Randomized trials and a meta-analysis comparing the same chemotherapy regimen administered in the adjuvant *versus* the neoadjuvant setting have demonstrated no difference in survival outcomes between the two strategies.[3–12] Therefore, there is current consensus that NAT represents at least an equivalent option to adjuvant treatment.[1,13] Notably, the neoadjuvant scenario represents a unique opportunity for research purposes: tumor and blood samples can be obtained at baseline, during NAT and at surgery, providing material to study predictive biomarkers and potential mechanisms of treatment resistance at different moments.[14]

A subset of the patients who receive NAT will achieve a pathologic complete response (pCR), defined as no residual invasive disease in the breast and the axillary lymph nodes, with rates varying according to the different breast cancer (BC)

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subtypes [hormone receptor-positive and human epidermal growth factor receptor 2 (HER2)-negative 7–16%; hormone receptor-positive and HER2-positive 30–40%; hormone receptor-negative and HER2-positive 50–70%; triple-negative BC (TNBC) 25–33%].[1,15–17] A 2014 meta-analysis including 12 trials and 11,955 patients confirmed the important prognostic value of pCR: patients achieving a pCR after NAT had a 56% reduction in the risk of recurrence in comparison with those not achieving a pCR.[18] The association between pCR and recurrence-free survival (RFS) and overall survival (OS) was significant for patients with TNBC and for those with HER2-positive, hormone receptor-negative BC. In hormone receptor-positive low-grade (grades 1 and 2) patients, the positive prognostic value of the pCR was not demonstrated.[18]

The presence of residual disease after NAT indicates the existence of partial treatment resistance in the tumor.[17,19] Many strategies have been explored to improve pCR rates and survival outcomes of BC patients, such as dose-intensification of NAT, addition of new drugs, extended treatment duration, and concomitant chemoradiation, without significant improvements in OS.[20–25] Most of the patients treated with NAT will not achieve a pCR and efforts to improve these results are necessary.[1,18] A potential strategy to overcome treatment resistance is to offer additional adjuvant treatment for patients that do not achieve a pCR after NAT, an approach described as 'post-neoadjuvant treatment'. The present manuscript comprises a review of the current literature on this strategy, including its rationale, the currently available post-neoadjuvant therapies, the ongoing trials evaluating new strategies and the translational research involving the residual disease to identify potential predictive and prognostic biomarkers, as well as potential targets for 'salvage therapy'.

Rationale for adapting NAT according to clinical response

Imaging studies and physical examination can be performed during NAT to obtain an early assessment of response. The objective of this strategy is to identify patients who are not responding to treatment, providing an opportunity for these individuals to receive agents with different mechanisms of action, in an attempt to overcome resistance. Studies investigating this strategy aimed to improve the pCR rates after NAT and were the pioneers for the development of the post-neoadjuvant treatment rationale.[26] Two main randomized trials have investigated the benefit of modifying ongoing NAT after an early assessment of clinical response.

In the GeparTrio trial, 2072 patients with operable or locally advanced BC had response assessments after two cycles of TAC (docetaxel 75 mg/m², doxorubicin 50 mg/m² and cyclophosphamide 500 mg/m² at D1, every 3 weeks). A total of 622 patients who did not present a response according to breast clinical examination and ultrasound (defined as a decrease in tumor size ⩾50%), were randomized 1:1 to proceed with either four cycles of TAC or change to four cycles of NX (vinorelbine 25 mg/m² D1 and D8, capecitabine 1000 mg/m² twice a day on D1–D14, every 3 weeks). Compared with the control arm assigned to TAC, patients who were switched to NX failed to achieve increased clinical response rates (50.5% *versus* 51.2%) or pCR rates (6% *versus* 5.3%).[27] Interestingly, updated results from this trial demonstrated a disease-free survival (DFS) benefit for early nonresponders assigned to TAC-NX *versus* those who continued TAC (hazard ratio [HR] 0.59; $p = 0.001$), although this was a secondary endpoint of the study.[28]

In the study by Smith and colleagues, 162 locally advanced BC patients started NAT with four cycles of CVAP (cyclophosphamide 1000 mg/m², doxorubicin 50 mg/m² and vincristine 1.5 mg/m² at D1, and prednisolone 40 mg/day D1–D5, every 21 days for four cycles), with the responders (clinical response assessed by physical examination) being randomized to four additional cycles of CVAP or four cycles of docetaxel 100 mg/m² every 21 days, while the nonresponders were switched to four cycles of docetaxel. A 55% clinical response rate was observed for patients who were not responding to CVAP and changed to docetaxel. However, the pCR rate in this group was only 2%, demonstrating that the switch to docetaxel was unable to increase pCR rates in patients that were not responding to an anthracycline-based chemotherapy.[29]

These trials demonstrated that the chances of achieving a pCR by chemotherapy modification according to response assessments during NAT are modest. The benefit of this strategy has not been confirmed in clinical trials and it is not routinely used. However, these studies have modified treatment according to clinical response assessed

by physical examination or ultrasound, which is not precise and presents a poor correlation with pathological response.[30,31] The interest in overcoming BC resistance by offering additional treatments with different mechanisms of action remained, but instead of relying on clinical response, a reasonable alternative would be to focus on the patients that do not achieve a pCR, thereby offering additional treatment for the individuals with demonstrated treatment resistance and a high risk of recurrence.

The post-neoadjuvant strategy

Most of the patients who receive NAT still present residual disease within the surgical specimen.[1,17] Considering the important prognostic value of achieving a pCR, by focusing on patients with suboptimal treatment response, the post-neoadjuvant setting represents the best scenario to select a population with a high recurrence risk. Several trials evaluating distinct post-neoadjuvant therapies such as additional chemotherapy with different agents, bisphosphonates, and poly ADP-ribose polymerase inhibitors (PARPis) failed to demonstrate a significant benefit (Table 1).

The first compelling data regarding post-neoadjuvant treatment came from the phase III CREATE-X trial. A total of 910 HER2-negative BC patients with residual invasive disease after NAT were randomized to receive capecitabine (1250 mg/m^2 twice per day D1–14, every 3 weeks for 6–8 cycles) plus standard of care (endocrine therapy for hormone receptor-positive patients and radiotherapy according to physician's discretion) *versus* standard of care alone. Initially, 159 patients received six cycles of capecitabine, but after an interim safety analysis, capecitabine treatment was extended to eight cycles. Endocrine treatment could be administered concomitantly to capecitabine, and radiotherapy was administered either before or after capecitabine. In this trial, a significant improvement in 5-year DFS from 67.6% to 74.1% in favor of capecitabine was observed (HR 0.70; 95% confidence interval [CI] 0.53–0.92, p = 0.01). The secondary endpoint of OS was also significantly improved: 5-year OS was 83.6% in the control *versus* 89.2% in the experimental arm (HR 0.59; 95% CI 0.39–0.90, p = 0.01).[36]

Subgroup analyses demonstrated a more pronounced benefit in TNBC patients in terms of DFS (56.1% in the control arm, *versus* 69.8% in the capecitabine arm; HR 0.58; 95% CI 0.39–0.87), and OS (70.3% in the control arm, *versus* 78.8% in the capecitabine arm; HR 0.52; 95% CI 0.30–0.90). In the hormone receptor-positive subgroup, the benefit did not reach statistical significance: DFS was 73.4% in the control arm *versus* 76.4% in the capecitabine arm (HR 0.81; 95% CI 0.55–1.17), and OS 90.0% *versus* 93.4% (HR 0.73; 95% CI 0.38–1.40). As expected, the most frequent grade 3–4 adverse events in the capecitabine arm were hand–foot syndrome (11.1%) and hematological toxicities (8.6%). Treatment completion with dose reductions was feasible in 23.9% of the patients assigned to six cycles and 36.7% of those assigned for eight cycles, while treatment interruption due to toxicities occurred in 18.2% of the patients assigned for six cycles and 25.4% of those assigned for eight cycles. No treatment-related deaths were recorded.[36]

The CREATE-X results are provocative, demonstrating a clinically meaningful survival improvement yielded by post-neoadjuvant capecitabine, which was relatively well tolerated. Most patients were able to complete post-neoadjuvant treatment, although dose reductions were frequent. Interestingly, the benefit was more pronounced in the TNBC subgroup, a population characterized by an elevated risk of recurrence and for whom there is an urgent need of new treatment strategies. Since pCR is a consistent prognostic factor and is associated with improved outcomes, by selecting individuals with residual disease, the CREATE-X trial may have offered additional treatment to those patients who really needed it, while sparing those who would not have benefited from this strategy.[18]

Notably, the addition of fluoropyrimidines to neoadjuvant/adjuvant treatments was investigated in several phase III trials, with most of them generating negative results (Table 2). Natori and colleagues performed a meta-analysis of randomized clinical trials evaluating the role of capecitabine in the neoadjuvant and adjuvant setting including eight studies and a total of 9302 patients. The addition of capecitabine to the chemotherapy regimen had no impact on DFS (HR 0.99; p = 0.93) nor on OS (HR 0.90; p = 0.36). A subgroup analysis demonstrated that the addition of capecitabine to standard chemotherapy was significantly associated with improved DFS in TNBC patients (HR 0.72; 95% CI 0.58–0.90, p = 0.02). Another meta-analysis from Zhang and colleagues including seven trials and 9097 patients also evaluated the addition of

Table 1. Clinical trials evaluating post-neoadjuvant treatment strategies.

Author	Year	Number of patients	Population	Treatment	Outcomes
Thomas, and colleagues[32] Phase II randomized	2004	193	T ⩾ 3 or N ⩾ 1, all BC subtypes	VACP × 3 ↓ Surgery Less than 1 cm^3 residual tumor: VACP × 5 More than 1 cm^3 residual tumor: A. vacp × 5 B. VbMF × 5	5-year RFS VACP 39% *versus* VbMF 49% ($p = 0.16$) 5-year OS VACP 47% *versus* VbMF 65% ($p = 0.20$)
Gonzalez-Angulo and colleagues[33] Phase II randomized	2015	43	HER2-negative BC with residual disease after NAT	A. Ixabepilone × 6 B. Observation	3-year RFS Ixabepilone 82% *versus* observation 94% ($p = 0.35$) Trial interrupted due to toxicities
Miller and colleagues BRE09-146[34] Phase II randomized	2015 (abstract)	128	Pts with *BRCA* mutation or TNBC with T > 2 cm or N ⩾ 1 in residual disease after NAT	A. Cisplatin × 4 B. Cisplatin × 4 plus Rucaparib for 36 weeks	2-year DFS Cisplatin 58.3% *versus* Cisplatin + Rucaparib 63.1% ($p = 0.43$)
von Minckwitz and colleagues[35] The NaTaN study Phase III	2016	693	All BC subtypes, residual disease after NAT	A. Zolendronate for 5 years B. Observation	5-year DFS Zolendronate 76.1% *versus* observation 75.1% ($p = 0.78$)
Masuda and colleagues[36] Phase III	2017	910	HER2-negative BC with residual disease after NAT	A. Capecitabine × 6 or × 8 + standard-of -care B. Standard of care (endocrine treatment and radiotherapy)	5-year DFS Capecitabine 74.1% *versus* control 67.6% ($p = 0.01$) 5-year OS Capecitabine 89.2% *versus* control 83.6% ($p = 0.01$)
von Minckwitz and colleagues[37] KATHERINE Phase III	2018	1486	HER2-positive BC with residual disease after NAT	A. TDM1 × 14 B. Trastuzumab × 14	3-year DFS TDM1 12.2% *versus* trastuzumab 22.2% ($p < 0.001$)

BC, breast cancer; DFS, disease-free survival; N, number of lymph nodes; NAT, neoadjuvant treatment; OS, overall survival; Pts, patients; RFS, recurrence-free survival; T, tumor size; TDM1, trastuzumab-emtansine; TNBC, triple-negative breast cancer; VACP, vincristine, doxorubicin, cyclophosphamide, and prednisone; VbMF, vinblastine, methotrexate, leucovorin and fluorouracil.

capecitabine into neoadjuvant and adjuvant chemotherapy regimens: whereas the addition of capecitabine had no impact on DFS (HR 0.93; 95% CI 0.85–1.02, $p = 0.12$), a significant improvement in OS was observed (HR 0.85; 95% CI 0.75–0.96, $p = 0.008$). Interestingly, in both meta-analyses the benefit of capecitabine was restricted to TNBC, in concordance with the most pronounced benefit observed in this subgroup in the CREATE-X trial.[36,38,39]

The distinct treatment concepts might explain the different results obtained: while in the neoadjuvant and adjuvant trials, capecitabine was administered to all patients combined with the chemotherapy regimen, in the CREATE-X the treatment was administered in the post-neoadjuvant setting exclusively to patients with residual disease. Also of note, the CREATE-X exclusively recruited Japanese and Korean patients. Asian individuals metabolize fluoropyrimidines more efficiently than Western

Table 2. Phase III trials investigating the addition of fluoropyrimidines to neoadjuvant or adjuvant chemotherapy.

Author	Year	Number of patients	Treatment	Outcomes
von Minckwitz and colleagues[23] GepparQuattro trial Phase III	2010	1421	**Neoadjuvant** EC × 4 Then randomized to: A. Docetaxel × 4 B. Docetaxel + capecitabine × 4 C. Docetaxel × 4 followed by capecitabine × 4	pCR rates A: 22.3% B: 19.5% C: 22.3% ($p = 0.29$)
Kelly and colleagues[40] Phase III	2012	601	**Neoadjuvant** A. Capecitabine + docetaxel × 4 followed by FEC × 4 B. Weekly paclitaxel × 12 followed by FEC × 4	4-year RFS A: 87.5% B: 90.7% ($p = 0.51$)
Ohno and colleagues[41] Phase III	2013	477	**Neoadjuvant** FEC × 4 Then randomized to: A. 4 cycles of docetaxel + capecitabine B. 4 cycles of docetaxel	pCR rates A: 23% B: 24% ($p = 0.74$)
Steger and colleagues[42] ABCSG-24 trial Phase III	2014	536	**Neoadjuvant** A. ET × 6 B. ET + capecitabine × 6	pCR rates A: 23% B: 15.4% ($p = 0.027$)
Bear and colleagues[24] NSABP B-40 NRG Oncology trial Phase III	2015	1206	**Neoadjuvant** A. Docetaxel × 4 B. Docetaxel + capecitabine × 4 C. Docetaxel + gemcitabine × 4 Followed by AC × 4	5-year DFS A: 72.8% B: 72.6% C: 73.9% ($p = 0.70$)
Martín and colleagues[43] GEICAM/2003-10 trial Phase III	2015	1384	**Adjuvant** A. EC + docetaxel × 4 B. ET + capecitabine × 4	5-year iDFS A: 86% B: 82% (HR 1.30; $p = 0.03$)
Del Mastro and colleagues[44] Phase III	2015	2091	**Adjuvant (factorial design)** A. FEC + paclitaxel q3 weeks B. FEC + paclitaxel q2 weeks (dose-dense) C. EC + paclitaxel q3 weeks D. EC + paclitaxel q2 weeks (dose-dense)	5-year DFS Fluorouracil *versus* no Fluorouracil (HR 1.06; $p = 0.561$)
Joensuu and colleagues[45] FinXX trial Phase III	2017	1500	**Adjuvant** A. Docetaxel + capecitabine × 3 followed by CEX × 3 B. Docetaxel × 3 followed by FEC × 3	10-year RFS A: 76.5% B: 78.5% (HR 0.88; $p = 0.23$)
Martín and colleagues[46] GEICAM/CIBOMA trial Phase III	2018	876	Adjuvant (after neoadjuvant chemotherapy and surgery) A. Capecitabine × 8 B. Observation	5-year DFS A. 79.6% B. 76.8% (HR 0.82; $p = 0.13$)

AC, doxorubicin and cyclophosphamide; CES, cyclophosphamide, epirubicin and capecitabine; CEX, cyclophosphamide, epirubicin and capecitabine; DFS, disease-free survival; EC, epirubicin and cyclophosphamide; ET, epirubicin and docetaxel; FEC, fluoracil, epirubicin and cyclophosphamide; HR, hazard ratio; iDFS, invasive disease-free survival; OS, overall survival; pCR, pathologic complete response; RFS, recurrence-free survival.

patients, with higher doses of capecitabine being more tolerable for this population.[47,48] In European and North American institutions, the regular capecitabine dose is 1000 mg/m² twice/day D1–14 every 21 days, in contrast with 1250 mg/m² used in the CREATE-X study.[49] These arguments raise concerns regarding the reproducibility of the results observed with capecitabine in Asian individuals in western patients.

Post-neoadjuvant treatment according to BC subtypes and ongoing trials

The CREATE-X results highlighted interesting points regarding the role of post-neoadjuvant treatment in BC. Over recent years, new therapies have emerged as effective or promising treatments in BC including the cyclin-dependent kinase inhibitors (CDKis), new estrogen receptor modulators, immunotherapy, PARPis, and androgen receptor antagonists.[50–55] Some of these strategies are being investigated in the post-neoadjuvant scenario or in combined adjuvant/post-neoadjuvant trials. In this section, we will discuss the available data and the ongoing studies evaluating post-neoadjuvant treatment in the different BC subtypes (Table 3 and Figure 1).

Post-neoadjuvant trials in TNBC

Additional chemotherapy. TNBC is associated with the worst prognosis and the highest risk of recurrence among all BC subtypes, although the response rates to chemotherapy in this subgroup are more pronounced than in hormone receptor-positive tumors: after NAT, pCR rates are around 33% in TNBC compared with 10% in hormone receptor-positive patients.[56,57] The role of additional chemotherapy as post-neoadjuvant treatment is under investigation in the ECOG-ACRIN Cancer Research phase III trial (ClinicalTrials.gov identifier: NCT02445391) in TNBC patients with residual invasive disease after taxane or anthracycline-based NAT. The trial initially randomized patients to post-neoadjuvant platinum chemotherapy (carboplatin or cisplatin) *versus* observation. After the publication of the CREATE-X study, this trial was amended, in order to interrupt observation and include capecitabine as a control arm. The rationale is to administer additional chemotherapy with different mechanisms of action in order to overcome resistance in patients with residual disease, the same as in the CREATE-X trial.[36]

PARPis. The use of a single-agent PARPi significantly improved progression-free survival compared with chemotherapy in previously treated metastatic BC patients harboring germline *BRCA* mutations.[58,59] In the post-neoadjuvant setting, the combination of cisplatin and the PARPi rucaparib has been investigated in the Hoosier Oncology Group BRE09-146 trial ($n = 128$).[34] Patients with *BRCA* mutations or TNBC with residual disease after NAT were randomized 1:1 to cisplatin (75 mg/m² D1 every 3 weeks for four cycles) ± rucaparib (24–30 mg D1–3 every 3 weeks for four cycles followed by 30 mg intravenously or 100 mg orally weekly for 24 weeks). DFS was similar in both arms (58.3% for cisplatin *versus* 63.1% for cisplatin + rucaparib; $p = 0.43$), and the *BRCA* status was not predictive of treatment benefit. Of note, the rucaparib dose in this trial was inferior to the 600 mg recommended phase II dose.[60] The phase III OLYMPIA trial is currently recruiting early HER2-negative BC patients harboring *BRCA* germline mutations to receive 1 year of adjuvant olaparib or placebo after surgery and neoadjuvant or adjuvant chemotherapy (ClinicalTrials.gov identifier: NCT02032823). The results of this study may clarify the pending questions regarding the activity of PARPis in TNBC patients, both in the adjuvant and the post-neoadjuvant scenarios.

Immunotherapy. Chemotherapy modulates the tumoral microenvironment by modifying the composition of stromal immune cells. A decrease in T regulatory and an increase in T cytotoxic cells in tissue and blood samples has been observed after NAT, suggesting an immune-modulatory effect of this treatment.[61,62] By reducing the population of regulatory T-cells and increasing the number of activated cytotoxic lymphocytes, NAT promotes an immune-activated tumoral microenvironment that may contribute to the efficacy of chemotherapy. The observation that elevated concentrations of tumor-infiltrating lymphocytes (TILs) in residual disease after NAT are associated with improved prognosis corroborates this hypothesis.[61,62]

The development of drugs that stimulate and promote an immune response against tumor cells yielded significant survival improvements in several diseases, such as melanoma and non-small cell lung cancer.[63–66] A high tumor mutational burden is a strong predictor of response to immunotherapy in non-small cell lung cancer, which might be explained by the fact that tumors harboring more

Table 3. Ongoing clinical trials investigating post-neoadjuvant treatment strategies.

Trial	Population	Rationale	Design	Treatment
TNBC				
ECOG-ACRIN NCT02445391	TNBC with residual invasive disease after NAT	Additional chemotherapy	Phase III	ARM A: cisplatin or carboplatin ARM B: capecitabine
NCT02530489	TNBC patients with residual disease after anthracycline-based NAT	Immunotherapy with anti-PDL1	Phase II	Weekly Nab-paclitaxel for 12 weeks plus atezolizumab (1200 mg every 3 weeks for 12 weeks) ↓ Surgery ↓ Atezolizumab four cycles
NCT02954874	TNBC patients with residual disease after NAT	Immunotherapy with anti-PD1	Phase III	ARM A: pembrolizumab for 12 months ARM B: placebo
NCT02926196	TNBC patients with residual disease after NAT	Immunotherapy with anti-PDL1	Phase III	ARM A: avelumab for 12 months ARM B: placebo
HER2-negative BC				
OLYMPIA NCT02032823	HER2-negative BC harboring *BRCA* mutation	PARPi	Phase III	ARM A: olaparib for 12 months ARM B: placebo
HER2-positive BC				
KATHERINE NCT01772472	HER2-positive BC who did not achieve pCR after NAT	TDM1	Phase III	ARM A: TDM1 for 14 cycles ARM B: trastuzumab for 14 cycles
NCT02297698	HER2-positive BC who did not achieve pCR after NAT	Vaccine combined with immune adjuvant	Phase II	Nelipepimut-S/GM-CSF for 2 years
NCT03384914	HER2-positive BC who did not achieve pCR after NAT	Comparison of two different vaccines (WOKVAC and DC1)	Randomized Phase II	ARM A: WOKVAC for 1 year ARM B: DC1 for 1 year
Hormone receptor-positive BC				
PENELOPE-B NCT01864746	Hormone receptor-positive, HER2-negative BC with residual disease after NAT	Combination of CDKi with endocrine treatment	Phase III	Standard endocrine therapy, and randomization to ARM A: palbociclib ARM B: placebo

BC, breast cancer; CDKi, cyclin-dependent kinase inhibitors; DC1, dendritic cell vaccine; NAT, neoadjuvant treatment; NCT, ClinicalTrials.gov identifier; PARPi, poly ADP-ribose polymerase inhibitor; pCR, pathologic complete response; PD1, programmed-death receptor 1; PDL1, programmed-death receptor ligand 1; TDM1, trastuzumab-emtansine; TNBC, triple-negative breast cancer.

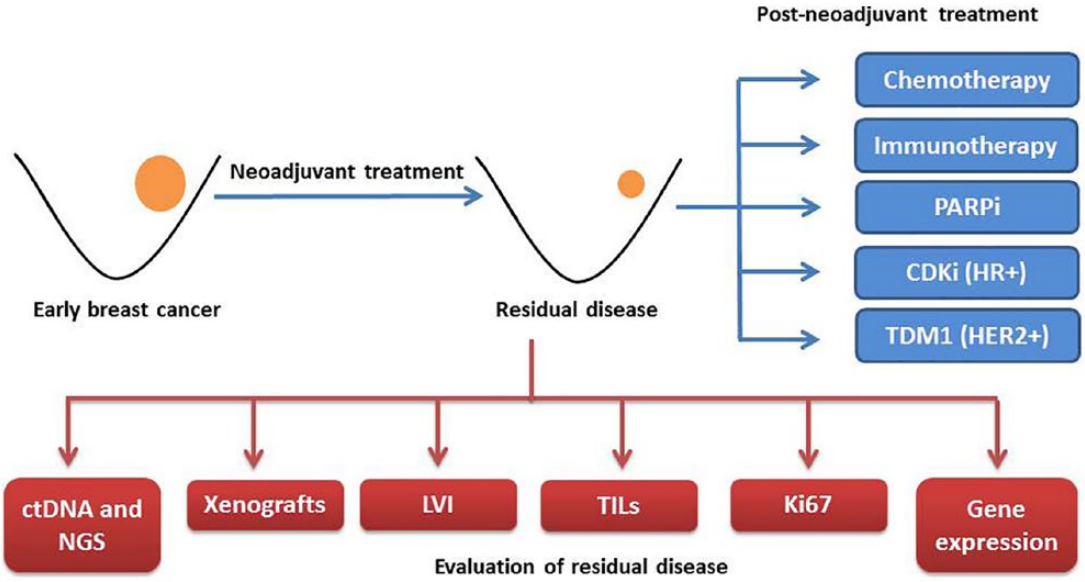

Figure 1. Potential strategies to manage residual disease after neoadjuvant treatment.
ctDNA, circulating tumoral DNA; HER2, human epidermal growth factor receptor 2; HR+, hormone receptor-positive breast cancer; LVI, lymphovascular invasion; NGS, next-generation sequencing; PARPi, poly ADP-ribose polymerase inhibitor; TDM1, trastuzumab-emtansine.

mutations express more aberrant proteins that can serve as antigens to be recognized by the immune system to induce an immune response.[67] There are promising ongoing trials exploring the role of immunotherapy as post-neoadjuvant treatment in BC, with most studies being focused on TNBC, which has the highest mutational burden among all BC subtypes and is also associated with the worst prognosis.[68–71]

Post-neoadjuvant trials in HER2-positive BC

Up to 66% of patients presenting HER2 amplification/overexpression treated with neoadjuvant chemotherapy plus dual HER2 blockade (pertuzumab and trastuzumab) are expected to achieve a pCR.[16,72] The current guidelines recommend maintaining anti-HER2 treatment after surgery in all patients to complete 1 year of treatment. However, the benefit of this consolidated strategy is uncertain for patients with residual disease, and it is also unknown whether patients who achieve a pCR with NAT need additional HER2 blockade after surgery.[73]

The ExteNET trial evaluated an extended adjuvant treatment with the pan-HER inhibitor neratinib in 2840 HER2-positive early BC patients, who were randomized to 1 year of neratinib *versus* placebo after the completion of adjuvant or neoadjuvant chemotherapy and trastuzumab for 1 year. Patients in the neratinib arm presented superior 5-year DFS rates when compared with placebo (90.2% *versus* 87.7%; HR 0.73; 95% CI 0.57–0.92, $p = 0.008$). The most frequent adverse events of grade 3 or higher in the neratinib arm were diarrhea (41%) vomiting (3%) and nausea (2%). Among the patients included in the study, 26% received NAT with chemotherapy and trastuzumab, and some of these patients probably did not achieve a pCR, meaning that patients with residual disease were included in the ExteNET trial. However, neratinib was administered after 1 year of trastuzumab treatment, and not as post-neoadjuvant treatment after surgery. Therefore, neratinib is an option of extended adjuvant treatment in HER2-positive patients, but given the lack of long-term OS data, the modest benefit and the high incidence of gastrointestinal toxicities observed in the ExteNET trial, neratinib may be considered in high-risk patients, particularly in hormone receptor-positive disease, and shall be evaluated on an individual basis, considering the expected benefit and the risk of toxicities.[74,75]

Trastuzumab-emtansine (TDM1), a drug-antibody conjugate formed by a monoclonal antibody against HER2 combined with the anti-microtubule chemotherapy agent DM1, has demonstrated activity in metastatic and early BC patients.[76,77]

To evaluate the activity of TDM1 in the post-neoadjuvant scenario, the KATHERINE phase III trial randomized 1486 patients with HER2-positive BC and residual disease after NAT to receive 14 cycles of TDM1 or to maintain trastuzumab for 14 cycles. In the interim analysis with a median follow up of 41.4 months, the 3-year invasive DFS rates were 88.3% in the TDM1 *versus* 77% in the trastuzumab group (HR 0.50; 95% CI 0.39–0.64, $p < 0.001$). The rate of distant recurrences was 10.5% with TDM1 *versus* 15.9% with trastuzumab, and all subgroups benefited from TDM1. Around 18% of the patients included in the study received NAT with trastuzumab and pertuzumab, the current standard of care for most HER2-positive patients.[37] Given the impressive results observed in the KATHERINE trial, post-neoadjuvant TDM1 represents the new standard of care treatment for HER2-positive patients with residual disease after NAT.

A phase II trial is evaluating nelipepimut-S/GM-CSF, a vaccine derived from the E75 peptide, an antigen expressed in the extracellular domain of HER2, combined with human granulocyte-macrophage colony-stimulating factor as an adjuvant to boost immune response. One of the trial's cohorts comprises patients who failed to achieve a pCR after a taxane and trastuzumab-containing NAT. Nelipepimut-S/GM-CSF will be administered for up to 2 years after the completion of the NAT. The primary endpoint of this study is invasive DFS (ClinicalTrials.gov identifier: NCT02297698). A randomized phase II trial is investigating two dendritic cell vaccines (WOKVAC and DC1) in HER2-positive BC patients with residual invasive disease after a trastuzumab-containing NAT. Patients are randomized 1:1 to DC1 or WOKVAC, with both vaccines being administered for 1 year, with the primary endpoint being DFS (ClinicalTrials.gov identifier: NCT03384914). The rationale is that the vaccines may be able to induce a T-cell-specific response against HER2-expressing cells, thereby inducing an anti-tumor immune response.

Post-neoadjuvant trials in hormone receptor-positive BC

The pCR rates in hormone receptor-positive BC after NAT are around 10%, much lower than the rates observed in HER2-positive and TNBC subtypes, thereby new strategies are necessary to improve pCR rates in this subgroup, even though the adjuvant endocrine therapy impacts significantly the long-term outcomes of this patients.[56] The cyclin-dependent kinases (CDKs) are serine–threonine kinases that regulate cell cycle progression from the G1 to the S-phase during mitosis.[78] CDKs activity can be abnormally increased or dysregulated in BC, leading to a constant stimuli for cell proliferation and survival, which is a known mechanism of resistance to endocrine treatment.[79] The CDK inhibitors (CDKis) target CDKs and block their activity, thereby restoring the regulation of the cell cycle.[79,80] In trials with metastatic hormone receptor-positive BC patients, the combination of a CDKis with first or second-line endocrine therapy yielded significant improvements in progression-free survival and response rates.[53,54,81–83] The role of CDKis in the post-neoadjuvant setting is being investigated in the phase III PENELOPE-B study. Hormone receptor-positive, HER2-negative patients who did not achieve a pCR after taxane-containing NAT receive standard endocrine therapy and are randomized 1:1 to palbociclib (125 mg once daily D1–D21 followed by 7 days off treatment in 28-day cycles) or 13 cycles or placebo (ClinicalTrials.gov identifier: NCT01864746).

As previously described, the OLYMPIA trial is recruiting BC patients harboring *BRCA* germline mutations to receive 1 year of adjuvant olaparib or placebo. This trial allows the inclusion of high-risk hormone receptor-positive patients ($\geqslant$4 lymph nodes in surgical specimen or residual disease after neoadjuvant treatment and a CPS + EG score $\geqslant$3).[84] By selecting patients with incomplete responses to neoadjuvant chemotherapy, this study will provide valuable data regarding the efficacy of a targeted therapy (PARPi) in the post-neoadjuvant setting for hormone receptor-positive high-risk patients harboring a *BRCA* mutation (ClinicalTrials.gov identifier: NCT02032823).

Perspectives

Potential biomarkers in residual disease

Expression of Ki-67. The protein Ki-67 is present in the nucleus of cells that are in mitosis, while it is not encountered in quiescent cells, thereby the expression of Ki-67 functions as a marker of cell proliferation.[85] A correlation between Ki-67 expression and the percentage of tumor cells that are in the S-phase of the mitotic cycle has been demonstrated.[86] The quantification of Ki-67 expression in tumor samples using immunohistochemistry provides a proliferative index, which is

an estimate of the proportion of the tumor cells that are in mitosis.[87] A direct correlation between Ki-67 expression and the pCR rates in BC patients has been demonstrated, with a high proliferative index being predictive of pCR.[88,89]

The expression of Ki-67 in surgical specimens of patients who did not achieve a pCR has been evaluated in exploratory studies. As shown in Table 4, which summarizes the results of these studies, a decrease in the proliferative index of the residual disease when compared with the pretreatment biopsy is a positive prognostic factor. On the contrary, a stable or increasing proliferative index in the residual disease is associated with worse outcomes. Therefore, the evaluation of Ki-67 expression at baseline and in residual disease may contribute to estimate the recurrence risks in patients receiving NAT. However, it is important to mention that Ki-67 expression in residual disease does not predict the benefit of additional treatments. Moreover, the studies that demonstrated the prognostic role of Ki-67 are mainly retrospective, and the validation of this biomarker in prospective trials is necessary.

Lymphovascular invasion. The presence of lymphovascular invasion (LVI) in tumor biopsies obtained before the administration of systemic treatment is a predictor of disease recurrence.[99,100] Hamy and colleagues have evaluated the prognostic impact of LVI in surgical specimens of 1033 BC patients after NAT. LVI was present in 29.2% of the samples analyzed, and was associated with worse DFS (HR 2.54; 95% CI 1.96–3.31; $p < 0.001$), with a more significant association observed in the triple-negative (HR 3.73; $p < 0.001$) and the HER2-positive (HR 6.21; $p < 0.001$) subtypes.[101] In another series of 166 early BC patients submitted to NAT, the frequency of LVI was 45% in residual disease samples, and the presence of LVI was significantly associated with worse DFS (HR 3.37; 95% CI 1.87–6.06, $p < 0.01$) and OS (HR 4.35; 95% CI 1.61–11.79, $p < 0.01$). Also in this study, the prognostic value of LVI for OS was more pronounced in TNBC (HR 6.06; 95% CI 2.08–17.68, $p < 0.001$).[102] Although the presence of LVI in residual disease is a negative prognostic factor that may contribute in the selection of high-risk patients to receive post-neoadjuvant treatment, LVI is not a predictor of the benefit of additional treatments. The role of LVI in residual disease should be further explored in studies evaluating post-neoadjuvant therapies and biomarkers in residual disease.

Scores evaluating the clinicopathological characteristics of residual disease. Since the presence of residual disease confers an increased risk of recurrence, a better characterization of these patients is necessary. The evaluation of the residual disease histologic parameters (such as nuclear grade, Ki-67 expression, LVI, hormone receptor and HER2 status), combined with pre-NAT clinical and pathological characteristics (tumor size, lymph node status, tumor cellularity) provides prognostic information. These features can be combined in the form of scores and algorithms, which can be used to estimate the risk of recurrence in this population.

The preoperative endocrine prognostic index (PEPI) score considers pre and post-NAT tumor size, Ki-67 expression levels, lymph node and hormone receptor status to estimate the risk of recurrence in hormone receptor-positive patients. The score categorizes patients into three groups (high, intermediate and low risk) according to the aforementioned clinical and pathological features. In two different cohorts, the PEPI score accurately predicted recurrence and BC-free survival, with significant differences in the recurrence rates between the three groups.[103] The CPS + EG score combines information of clinical and pathological stages with hormone receptor expression and nuclear grade in residual disease to estimate the risk of recurrence in hormone receptor-positive patients.[84] This score has demonstrated to be an accurate tool to predict prognosis, by dividing patients into six categories, with highest scores being associated with increased risks of recurrence. Other scores incorporating clinical and pathological variables have been developed to provide an estimation of the recurrence risks in patients with residual disease.[104,105] By providing an estimation of BC recurrence risks, these scores provide valuable prognostic information that can be used to guide therapeutic decisions, although they cannot predict the benefit of additional treatments.

Gene expression, proteomic and mutation profile in residual disease. There is growing interest in obtaining knowledge from residual disease and understanding the mechanisms involved in treatment resistance. The gene expression patterns in BC cells can be modified by NAT, with residual disease presenting different expression levels of genes involved in cell proliferation, invasiveness, cell cycle and immune response when compared with pretreatment tumor samples.[104,106,107] Similarly, the expression of certain proteins or the frequency of mutations such as TP53 and PI3K can

Table 4. Studies evaluating Ki-67 expression in residual disease.

First author	Number of patients	Ki-67 evaluation	Ki-67 cut-off	Results
Burcombe and colleagues[90]	27	Before NAT, after one cycle of NAT and at the surgical specimen	N/A	18 out of 26 patients had reduced Ki-67 levels in residual disease compared with baseline
Jones and colleagues[91]	103	Before NAT and at the surgical specimen	N/A	5-year RFS 27% for high Ki-67 77% for low Ki-67 (in residual disease)
von Minckwitz and colleagues[92]	667	Before NAT and at the surgical specimen	High >35%	High levels in residual disease associated with worse DFS (HR 4.53; $p < 0.001$)
Sheri and colleagues[93]	220	Before NAT and at the surgical specimen	High >17%	5-year RFS Low – 76% High – 33% ($p < 0.001$)
Yoshioka and colleagues[94]	64	Before NAT and at the surgical specimen	High >14%	High levels in residual disease associated with increased risk of recurrence (RR 69.23, $p = 0.003$)
Yamazaki and colleagues[95]	217	Before NAT and at the surgical specimen	High >20%	High levels in residual disease associated with increased risk of recurrence ($p = 0.022$)
Montagna and colleagues[96]	904	Before NAT and at the surgical specimen	High >20%	Ki-67 expression decrease associated with improved DFS (HR 0.52, $p < 0.001$)
Diaz-Botero and colleagues[97]	357	Before NAT and at the surgical specimen	High >15%	High levels in residual disease associated with increased risk of recurrence ($p < 0.001$)
Cabrera-Galeana and colleagues[98]	435	Before NAT and at the surgical specimen	Decrease ⩾1% in Ki-67 expression in residual disease versus no decrease or increase in Ki-67 in residual disease	Increased risk of recurrence for the patients with no decrease or increase in Ki-67 (HR 3.39, $p < 0.001$)

DFS, disease-free survival; HR, hazard ratio; NAT, neoadjuvant treatment; RFS, recurrence-free survival; RR, relative risk.

be affected by NAT.[106–108] As shown in Table 5, which summarizes the results of the main studies evaluating potential molecular biomarkers in residual disease, some of these changes have prognostic significance, while others have revealed potential treatment resistance mechanisms or potential future therapeutic targets.

While additional clinical validation is needed, the use of gene expression, proteomics and mutational profiles as predictive or prognostic biomarkers represents a promising strategy to be further evaluated in clinical trials with the objective of providing individualized therapies associated with less toxicities for BC patients. However,

Table 5. Studies evaluating molecular biomarkers in residual disease.

First author	*n*	Population	Biomarker / methods	Results
Gonzalez-Angulo and colleagues[107]	21	All BC subtypes	Gene expression PAM50	PAM50 subtype changed in 33.3% of the cases after NAT Increase in the expression of genes related to cell proliferation and reduction in genes involved in immune pathways
Dunbier and colleagues[109]	81	Hormone receptor-positive BC	Gene expression by Illumina	Downregulation of genes associated with cell proliferation and estrogen receptor Upregulation of cytokines and genes associated with immune response
Yu and colleagues[110]	1st cohort: 49 Validation cohort: 25	TNBC	Expression of seven genes (AR, GATA3, ESR2, GBX2, KRT16, MMP28, WNT11) to define high *versus* low risk profiles	3-year RFS 76.9% for low risk *versus* 25% for high risk (HR 4.67; 95% CI 1.27–17.15)
Balko and colleagues[111,112]	1st cohort: 49 Validation cohort 1: 89 Validation cohort 2: 74	TNBC	Multigene expression MCL1, MYC and JAK2 amplifications and mutations	Downregulation of DUSP4 (MAPK inhibitor) in residual disease Worse OS for JAK2 amplifications (HR 4.16; $p = 0.002$) and MAPK pathway activation ($p = 0.0013$)
Magbanua and colleagues[113]	39	All BC subtypes	Gene expression PAM50 before and after NAT	Upregulation of genes related to cytokines and cell proliferation in residual disease associated with reduced RFS
Klintman and colleagues[114]	126	All BC subtypes	Expression of 24 genes associated with cell proliferation and treatment resistance	6-gene signature (ACACB, CD3D, DECORIN, ESR1, MKI67, PLAU) associated with worse prognosis ($p = 0.0067$)
Beitsch and colleagues[115]	93	All BC subtypes	Gene expression MammaPrint	16 patients went from high risk to low risk, 1 changed from low risk to high risk, and 4 patients have changed their molecular subtype but remained in the high-risk population MammaPrint scores significantly altered after NAT ($p = 0.001$)
Pinto and colleagues [116]	1st cohort: 82 patients Validation cohort: 113 patients	TNBC	Gene expression (449 genes profiled with NanoString)	3-gene signature (CCL5, DDIT4 and POLR1C) associated with DFS outcomes, with high-risk predicting worse prognosis ($p = 0.002$)
Gay-Bellile and colleagues[117]	90	All BC subtypes	ERCC1 (gene and protein expression, gene-copy number) Telomeres stability (expression of genes associated with telomere function)	ERCC1 copy number variations associated with worse DFS ($p = 0.026$) and OS ($p = 0.043$) Telomere instability/dysfunction associated with worse prognosis (HR 5.41 for DFS; $p = 0.0044$)
Gonzalez-Angulo and colleagues[118]	1st cohort: 79 Validation cohort: 99	1st cohort All BC subtypes Validation cohort Hormone receptor-positive BC	Reverse phase protein arrays	1st cohort: 3 protein-score (phospho-CHK, caveolin-1and RAB25) to define low *versus* high-risk 3-year RFS 66.8% *versus* 45.1% ($p = 0.002$) 2nd cohort cyclin E1 and CD31 to define low *versus* high-risk. 3-year RFS 90.8% *versus* 16.7% ($p \leqslant 0.001$)
Sohn and colleagues[119]	54	TNBC	Reverse phase protein arrays	5 protein-score (AKT, IGFBP2, LKB1, S6 and Stathmin) to define low *versus* high-risk 3-year RFS 48.4% *versus* 7.14% ($p = 0.001$)
Yuan and colleagues[120]	102	All BC subtypes	Polymerase chain reaction PI3K mutations	Trend to worse DFS for patients who maintained PI3K mutation in residual disease ($p = 0.052$)
Jiang and colleagues[106]	1st cohort: 206 2nd cohort: 158	All BC subtypes	Sanger sequencing DNA–PI3K and TP53 mutations	Improved DFS ($p = 0.033$) and OS ($p = 0.045$) in patients with TP53 or PI3K loss after NAT

BC, breast cancer; CI, confidence interval; DFS, disease-free survival; ERCC1, excision repair cross complementation group 1 protein; HR, hazard ratio; N, number of patients; NAT, neoadjuvant therapy; OS, overall survival; RFS, recurrence-free survival; TNBC, triple-negative breast cancer.

potential limitations to implement these strategies exist. Biomarker evaluation requires tissue sampling (often with multiple biopsies) and complex molecular techniques, which may be difficult to perform in low-resource scenarios. Despite the interesting results observed with biomarker-driven therapy, validation in prospective large cohorts is necessary before the incorporation of these tests into clinical practice.

Circulating tumoral DNA. Another way of studying the tumor mutational profile is through the isolation of circulating tumoral DNA (ctDNA) in plasma, with the advantage that this technique does not require an invasive tissue biopsy.[121] Garcia-Murillas and colleagues reported interesting data on 55 BC patients that, after completing NAT and surgery, were screened for the presence of ctDNA in plasma samples during their follow up. The presence of ctDNA was significantly associated with disease recurrence (HR 25.1; 95% CI 4.08–130.5, $p < 0.0001$).[122] Of note, there was a median lead time of 7.9 months between ctDNA detection and clinical recurrence, demonstrating the potential of ctDNA as an early predictor of recurrence in the follow up of BC patients. The sensitivity of the ctDNA technique can increase significantly when multiple and consecutive samples are analyzed, therefore the fact that this study collected periodic samples during patient's follow up may have contributed to the results observed.[122,123]

Chen and colleagues evaluated the mutational profile of 38 TNBC patients with residual disease after NAT using a multigene panel with next-generation sequencing. A total of 33 out of 38 samples analyzed presented at least one somatic mutation, with TP53 (82%), PI3K (16%) and AKT1 (5%) being the most frequent. Further, ctDNA isolated from plasma samples obtained after surgery was tested for the same mutations present in the tumors. Of the 33 patients who harbored a somatic mutation in residual disease, 4 presented the same mutation in ctDNA. Interestingly, the recurrence rates were 100% among patients with detectable ctDNA after NAT, *versus* 26% in patients with undetectable ctDNA levels. Patients with detectable ctDNA after surgery had inferior DFS when compared with those with undetectable ctDNA: median DFS 4.6 months *versus* not reached (HR 12.6; 95% CI 3.06–52.2, $p < 0.001$).[124] In this study, the detection of ctDNA after NAT was significantly associated with an increased risk of recurrence, although the exploratory nature of the analysis and its limited sample size do not allow definitive conclusions.

The assessment of ctDNA after NAT in patients with residual disease provides valuable information regarding the tumor mutational profile of chemoresistant cells, which will probably be involved in disease recurrence. Therefore, this strategy can potentially improve the detection of disease recurrence and also guide the selection of patients with the highest risk of recurrence as potential candidates for additional treatments or for the inclusion in clinical trials. The role of ctDNA as a prognostic and predictive biomarker in the post-neoadjuvant setting needs validation in prospective clinical trials before being incorporated into clinical practice.

TILs and biomarkers of immune activation. The presence of TILs in biopsy samples has been associated with improved prognosis in several cancers, including BC.[125–129] The levels of TILs can change significantly during NAT.[130,131] Besides its prognostic significance, there is also interest about the role of TILs in residual disease. One of the postulated mechanisms by which chemotherapy exerts its anti-tumoral effects is the induction of an immune response against the tumor, mediated by dendritic cells and cytotoxic lymphocytes that become activated by the antigen exposure resulting from cell death and apoptosis.[102,103,132–134]

In a secondary analysis of the NeoALTTO trial, Salgado and colleagues evaluated TIL levels at baseline in 387 HER2-positive early BC patients treated with NAT (trastuzumab, lapatinib, or both for 6 weeks, then weekly paclitaxel was associated for 12 weeks, followed by three cycles of adjuvant fluorouracil, epirubicin, and cyclophosphamide). TIL levels higher than 5% were associated with increased pCR rates (OR 2.60; 95% CI 1.26–5.39, $p = 0.01$), and each 1% increase in TIL level yielded a 3% decrease in the recurrence rates (HR 0.97; 95% CI 0.95–0.99, $p = 0.002$).

Intriguingly, a study by Hamy and colleagues evaluating TIL levels before and after NAT with chemotherapy and trastuzumab in 175 HER2-positive BC patients demonstrated that TIL levels decreased in 78% of the patients during NAT, and that the TIL level decrease was predictive of a pCR ($p < 0.001$). The baseline TIL levels were not associated with prognosis, although in the

population of patients with residual disease ($n = 107$), TIL levels higher than 25% were associated with worse survival (HR 7.89; 95% CI 1.68–37.77, $p = 0.009$). This study highlights that the role of TILs in residual disease needs to be further investigated, and the interpretation of TIL levels might differ according to BC subtype and the use of targeted therapies (such as anti-HER2 treatment).[130]

NAT also promotes a modification in the tumoral microenvironment by reducing the concentration of CD4+ T-cells, which are immunomodulatory, while increasing the concentration of CD8+ T-cells, which are cytotoxic and have anti-tumoral activity.[135] The final effects of these modifications induced by NAT are a tumoral microenvironment that is more inflammatory and immunogenic. A high infiltration of CD8+ lymphocytes in the tumor is also associated with increased pCR rates, suggesting that a proinflammatory microenvironment is an important component of the anti-tumor activity of cancer treatments.[135]

The transcription factor FOXP3 is expressed in a subset of T lymphocytes that exert an immunomodulatory and suppressive effect on the immune response.[136] Ladoire and colleagues have developed a score based on the ratio between CD8+ and FOXP3+ lymphocyte concentrations in the tumoral microenvironment of BC patients, comprising one discovery cohort (111 HER2-positive patients) and two separate validation cohorts (1st cohort: 84 HER2-positive patients; 2nd cohort: 51 HER2-negative patients). Higher ratios (indicative of a more immunogenic tumoral stroma) were significantly associated with improved prognosis. In the subgroup of HER2-positive patients, the score was more accurate than pCR for predicting OS.[137] Liu and colleagues also evaluated FOXP3+ cells in the tumoral microenvironment in 132 patients (111 of which had residual disease samples). A high concentration of FOXP3+ cells in residual disease was associated with worse DFS ($p = 0.006$) and OS ($p = 0.001$).[138] The same results were described in a series of 131 TNBC patients by Miyashita and colleagues, who assessed the CD8/FOXP3 ratio at baseline and in residual disease. The 5-year DFS rates were 72% for patients with a high CD8/FOXP3 ratio, compared with 40% in patients with a low CD8/FOXP3 ratio ($p = 0.009$).[139] These results highlight the role of immune activation in the tumoral stroma as a prognostic factor but also as a potential therapeutic target to be explored.

Although the prognostic value of TILs in residual disease has been demonstrated, most of the studies were small and retrospective, with significant discrepancies between the methodologies used to quantify TILs. In 2017, the International Immuno-Oncology Biomarker Working Group on Breast Cancer published a recommendation for the use of TILs in BC, including specifications for residual disease, describing standard criteria for TIL quantification.[140] The implementation of these guidelines in routine pathology assessment should allow an improvement in the consistency of TIL evaluation, which can become an interesting stratification factor for future clinical trials.

Patient-derived xenografts and experimental in vivo *models with residual disease*

The implant of BC cells into immune-deficient mice constitutes a xenograft, and when tissue derived from a patient's tumor is implanted, then it is defined as a patient-derived xenograft.[141] This technique provides an opportunity to evaluate *in vivo* the tumor sensitivity to anticancer drugs, to study tumor growth and the tumor molecular profile at different moments.[142] Goetz and colleagues developed xenografts from 140 early BC patients with biopsies obtained before NAT and at surgery, with samples being analyzed with a next-generation sequencing multigene panel. No differences were observed in the number of targetable mutations of the pre-NAT *versus* the residual disease samples. Interestingly, in one patient who progressed during NAT, a *BRCA1* mutation was identified. In this case, investigators demonstrated the anti-tumoral activity of olaparib in pre-NAT sample xenografts, and also in the xenografts from the residual disease.[143] Another study with 48 early BC samples (24 obtained before NAT and 24 from residual disease) has performed multigene sequencing of xenografts and compared the results with the baseline tumoral samples. The main somatic mutations were maintained between xenografts and tumor samples before and after NAT, suggesting that xenografts preserve the genetic characteristics of the original BC samples.[144]

Zhang and colleagues developed 13 xenograft models derived from tumor samples obtained from BC patients before NAT, to compare the treatment responses observed in the xenografts with the responses observed in patient's tumors. In 92% of the cases, the xenograft reproduced the pattern of response observed in the primary

tumor.[145] Yu and colleagues also observed that xenografts exposed to paclitaxel reproduced exactly the patients' clinical response in patient-derived xenograft models.[146] These results suggest that xenografts can reproduce treatment responses observed in the primary tumor, and serve as potential models to test different treatments and estimate their effects *in vivo*.

Marangoni and colleagues developed xenografts from samples of residual disease in 10 TNBC patients that did not achieve a pCR after NAT, aiming to evaluate the sensitivity to different drugs and seek for predictive biomarkers in this scenario. All patients received taxanes, eight received anthracyclines and six received fluorouracil as part of the NAT. The sensitivity of the residual disease xenografts to different agents was evaluated: capecitabine, anthracyclines combined with cyclophosphamide, taxanes and platins. Capecitabine induced tumor shrinkage in 60% of the xenografts, while the response rate to other drugs was modest (10% for doxorubicin and cyclophosphamide, 20% for cisplatin and 0% for docetaxel). Interestingly, a high expression of RB1 (retinoblastoma-associated protein, involved in cell cycle regulation) and TYMP (thymidine phosphorylase, an enzyme that acts in the conversion of capecitabine to 5-fluorouracil) were predictive of capecitabine response.[147] The results observed in xenografts reproduced the sensitivity of TNBC residual disease to capecitabine observed in the CREATE-X trial, and identified potential predictive biomarkers to be explored in future studies.[36]

The studies with xenografts demonstrate that the tumoral mutational profile and the patterns of gene expression are very similar between baseline tumors and residual disease. Moreover, there is a high concordance between treatment responses observed in patients and their respective xenograft models. Xenografts derived from residual disease provide an opportunity to study treatment resistance mechanisms, to explore new therapeutic targets and to evaluate drug responses *in vivo*. Patient-derived xenograft models have a huge potential to be further explored, and may become a valuable tool to guide individualized treatment for BC patients in the post-neoadjuvant setting. There are ongoing trials developing xenografts from residual disease, aiming to characterize its molecular profile and evaluate new therapeutic agents in these models (ClinicalTrials.gov identifiers: NCT02247037, NCT02732860).

Conclusion

The interest in post-neoadjuvant treatment has increased after the publication of the CREATE-X trial and its positive results, and the addition of capecitabine is now considered a valuable option for patients with residual disease after NAT, particularly for the TNBC subtype, for which the magnitude of the benefit was more pronounced. Uncertainty remains if the CREATE-X results are reproducible in western patients and if lower doses of capecitabine will produce the same results. Also, the negative trials evaluating the addition of capecitabine/5-fluorouracil to neoadjuvant and adjuvant treatments conflict in part with the results observed on the CREATE-X trial, raising questions about the real benefit of capecitabine as post-neoadjuvant treatment. Nevertheless, the proper selection of the high-risk patients with residual disease after NAT may explain the differences observed between trials and explain the positive results of 'CREATE-X'. The 2018 guidelines from the American Society of Clinical Oncology state that capecitabine can be administered to HER2-negative BC patients with invasive residual disease after NAT (intermediate evidence quality).[75]

The robust DFS improvement observed in the KATHERINE study supports the incorporation of TDM1 as a new standard treatment for HER2-positive patients presenting residual disease after NAT with chemotherapy and anti-HER2 treatments, although a longer follow up will allow a more precise estimation of the OS impact and the potential long-term toxicities of this strategy. As occurred in the CREATE-X trial, the selection of high-risk patients may have contributed to increase the magnitude of benefit observed with the post-neoadjuvant treatment.

Several promising strategies are currently being evaluated in the post-neoadjuvant setting, such as immunotherapy and targeted therapies. A more precise knowledge of the tumor's molecular and genomic characteristics will certainly improve the development of clinical trials for the treatment of residual disease. The advent of new technologies such as DNA sequencing, gene expression profiles and patient-derived xenografts has improved our understanding of tumor biology, of the pathways involved in tumor progression and of drug resistance mechanisms.[148] The characterization of the tumor molecular profile and its interaction with the tumoral microenvironment in each BC patient will refine therapy by guiding treatment individualization. Moreover, a better understanding of

'residual disease', with the identification and validation of prognostic and predictive biomarkers, will contribute to the identification of patients who will truly benefit from additional treatment. The CREATE-X and the KATHERINE studies represent the beginning of a promising era of novel post-neoadjuvant treatment strategies in BC.

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