Cancer Treatment Reviews 132 (2025) 102852

Contents lists available at ScienceDirect

Cancer Treatment Reviews

journal homepage: www.elsevier.com/locate/ctrv

Hot Topic

Estrogen Signaling in Early-Stage Breast Cancer: Impact on Neoadjuvant Chemotherapy and Immunotherapy

Chiara Corti [a,b,c,d,*], Busem Binboğa Kurt [a,b,e], Beyza Koca [a,b], Tasnim Rahman [a,b], Fabio Conforti [f], Laura Pala [f], Giampaolo Bianchini [g,h], Carmen Criscitiello [c,d], Giuseppe Curigliano [c,d], Ana C. Garrido-Castro [a,b], Sheheryar K. Kabraji [i], Adrienne G. Waks [a,b], Elizabeth A. Mittendorf [a,b,j,1], Sara M. Tolaney [a,b,1]

[a] Breast Oncology Program, Dana-Farber Brigham Cancer Center, Boston, MA, USA
[b] Harvard Medical School, Boston, MA, USA
[c] Division of New Drugs and Early Drug Development for Innovative Therapies, European Institute of Oncology, IRCCS, Milan, Italy
[d] Department of Oncology and Hematology-Oncology (DIPO), University of Milan, Milan, Italy
[e] Division of Pathology, Brigham and Women's Hospital, Boston, MA, USA
[f] Department of Medical Oncology, Humanitas Gavazzeni, Bergamo, Italy
[g] Department of Medical Oncology, San Raffaele Hospital, IRCCS, Milan, Italy
[h] School of Medicine and Surgery, Vita-Salute San Raffaele University, Milan, Italy
[i] Department of Medicine, Molecular and Cellular Biology, Roswell Park Comprehensive Cancer Center, Buffalo, NY, USA
[j] Division of Breast Surgery, Brigham and Women's Hospital, Boston, MA, USA

ARTICLE INFO

Keywords:
Breast cancer
Immunotherapy
Microenvironment
Prediction
Estrogen receptor
Neoadjuvant
Neoadjuvant chemotherapy
Neoadjuvant chemoimmunotherapy

ABSTRACT

Neoadjuvant chemoimmunotherapy (NACIT) has been shown to improve pathologic complete response (pCR) rates and survival outcomes in stage II-III triple-negative breast cancer (TNBC). Promising pCR rate improvements have also been documented for selected patients with estrogen receptor-positive (ER+) human epidermal growth factor receptor 2-negative (HER2-) breast cancer (BC). However, one size does not fit all and predicting which patients will benefit from NACIT remains challenging. Accurate predictions would be useful to minimize immune-related toxicity, which can be severe, irreversible, and potentially impact fertility and quality of life, and to identify patients in need of alternative treatments.

This review aims to capitalize on the existing translational and clinical evidence on predictors of treatment response in patients with early-stage BC treated with neoadjuvant chemotherapy (NACT) and NACIT. It summarizes evidence suggesting that NACT/NACIT effectiveness may correlate with pre-treatment tumor characteristics, including mutational profiles, ER expression and signaling, immune cell presence and spatial organization, specific gene signatures, and the levels of proliferating versus quiescent cancer cells.

However, the predominantly qualitative and descriptive nature of many studies highlights the challenges in integrating various potential response determinants into a validated, comprehensive, and multimodal predictive model. The potential of novel multi-modal approaches, such as those based on artificial intelligence, to overcome current challenges remains unclear, as these tools are not free from bias and shortcut learning. Despite these limitations, the rapid evolution of these technologies, coupled with further efforts in basic and translational research, holds promise for improving treatment outcome predictions in early HER2- BC.

Introduction

Breast cancer (BC) affects 1 in 8 women and is one of the leading causes of cancer-related deaths worldwide [1]. Almost 10–15% of early BCs lack expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2), therefore they are referred to as triple-negative breast cancers (TNBCs). In TNBC, pathologic complete response (pCR) to neoadjuvant therapy is linked to

* Corresponding author.
E-mail address: chiara_corti@dfci.harvard.edu (C. Corti).
[1] co-senior author.

https://doi.org/10.1016/j.ctrv.2024.102852
Received 10 September 2024; Received in revised form 14 October 2024; Accepted 10 November 2024
Available online 13 November 2024

a lower risk of recurrence [2–4]. Recently, the addition of the anti-programmed cell death protein 1 (PD-1) monoclonal antibody pembrolizumab, an immune checkpoint inhibitor (ICI), to neoadjuvant chemotherapy (NACIT) has become standard of care in early high-risk TNBC [3–5]. This indication is based on the results of the phase 3 KEYNOTE-522 randomized controlled trial (RCT), which investigated neoadjuvant paclitaxel, carboplatin, doxorubicin/epirubicin and cyclophosphamide plus pembrolizumab/placebo, followed by surgery, and then adjuvant pembrolizumab/placebo in stage II-III TNBC [3,4]. The trial demonstrated at a pre-planned interim analysis that the addition of pembrolizumab to neoadjuvant chemotherapy (NACT) raised the overall pCR rate from 51% to 65% [4]. At a median follow-up of 63.1 months, the addition of pembrolizumab improved event-free survival (EFS, 81.3 % with pembrolizumab versus 72.3 % with placebo), with a 37% reduction in events (hazard ratio [HR] 0.63, 95% CI, 0.49–0.81) [6]. Importantly, at a median follow-up of 75.1 months, a statistically significant and clinically meaningful benefit in overall survival (OS) estimated at 60 months was found (86.6% with pembrolizumab versus 81.7% with placebo, p = 0.002) [7]. The impact of the addition of ICI to NACT in TNBC has been investigated also by other clinical trials, as summarized in Table 1.

The Alexandra/IMpassion030 trial assessed the efficacy of administering ICI in combination with chemotherapy (CT) in the adjuvant setting after upfront surgery, with disappointing results (futility was declared based on HR 1.12; 95% CI, 0.87–1.45, p = 0.370), with no differences seen in any subgroup including PD-L1 status [20]. This

Table 1
Selected phase 2–3 clinical trials which investigated the impact of immune checkpoint inhibitors in early triple-negative breast cancer.

Study, Immunotherapy agent (target)	Chemotherapy regimen	Patient population	Subgroup	Immunotherapy Number	Immunotherapy pCR (%)	Control arm Number	Control arm pCR (%)	EFS results (exp. arm versus control)
Neoadjuvant and adjuvant immunotherapy								
KEYNOTE-522, Pembrolizumab (PD-1, 22C3) [3,4,6]	P/Cp + AC	~25% stage III; ~50 % N+	All	784	65*	390	51*	mFUP 63.1 months, EFS 81.3% vs 72.3% (HR 0.63, 95% CI, 0.49–0.81)
			PD-L1+	656	69	317	55	
			PD-L1–	127	45	69	30	
Impassion031, Atezolizumab (PD-L1, SP-142) [8]	Nab-P + ddAC	~23% stage III 38% N+	All	165	58	168	41	mFUP ~ 20 months, EFS not reached (HR 0.76, 95% CI, 0.4–1.44)
			PD-L1+	77	69	75	49	
			PD-L1–	88	48	93	34	
Only neoadjuvant immunotherapy								
NeoTRIPaPDL1, Atezolizumab (PD-L1, SP142) [9,10]	Cp + Nab-p	~50% locally advanced; ~88% N+	All	138	49	142	44	mFUP 54 months, EFS 70.6% vs 74.9% (HR 1.06, 95% CI, 1.076 (0.670 1.731)
			PD-L1+	79	60	77	52	
			PD-L1–	59	34	65	35	
GeparNUEVO, Durvalumab (PD-L1, SP263) [11,12]	Nab-P + ddAC	65% stage ≥ IIA; ~30% N+	All	88	53	86	44	mFUP 42 months, 3-year iDFS 85 vs 77% (HR 0.54, 95% CI 0.27–1.09)
			PD-L1+	69	58	69	51	
			PD-L1–	11	44	9	18	
			Window[#]	59	61	58	41	
NeoPACT[§], pembrolizumab (PD-1, 22C3) [13]	Cp + D	~75% stage II ~13% stage III	All	111	58	NA	NA	3-year EFS, 86% in all patients; 98% in pCR group and 68% in no-pCR group (HR, 0.057, 95 % CI 0.01–0.45)
			PD-L1+	52	75	NA	NA	
			PD-L1–	60	40	NA	NA	
NeoMono, atezolizumab[$] (PD-L1, SP142) [14]	P/Cp + AC	NA	All	Window arm: 180	65.7	No window arm: 179	69	NA
			PD-L1+	NA	91	NA	82	
			PD-L1–	NA	56	NA	64	
Neo-N, nivolumab lead-in[°] (PD-1) [15]	P/Cp	~65% stage II-III	All	Lead-in arm: 53	51	Concurrent arm: 55	54	NA
I-SPY2, pembrolizumab arm (PD-1, 22C3) [16]	P + AC	~42% palpable N (ER+/ER-)	All	69	60	181 (rolling control)	22	HR, 0.60 (no CI reported)
I-SPY2, durvalumab + olaparib arm (PD-L1, SP263) [17]	P + AC	~30% palpable N (ER+/ER-)	All	20	47	130 (rolling control)	27	NA
I-SPY2.2, datopotomab-deruxtecan and durvalumab [18]	Block B (paclitaxel, carboplatin, pembrolizuman) and/or Block C (AC and pembrolizumab)	Mammaprint high risk, stage II-III, (approximately 2/3 ER-)	HER2– Immune+	47	65.0	NA	NA	NA
BELLINI, cohort C, ipilimumab (CTLA-4) + nivolumab (PD-1) [19]	None	Stage I-II, N0, TILs ≥ 50%	Cohort C, all	15	33 (5/15)	NA	NA	NA

* The pCR values listed for KEYNOTE-522 comes from the initial presentation of results for the first 602 patients. When results for all patients were available, final pCR rates were 63 and 56% for pembrolizumab and placebo, respectively. # The first 117 patients treated on GeparNeuvo received a single dose of either durvalumab or placebo two weeks before starting neoadjuvant chemotherapy with the same agent; this was omitted after the study's independent data monitoring committee expressed concern about the delay in the start of chemotherapy; another 57 patients were subsequently treated without this "window" treatment. § In NeoPACT, 16% of enrolled patients had low ER − PR (1%-10%); $, arm A, atezolizumab monotherapy window, then atezolizumab with chemotherapy, arm B, atezolizumab with chemotherapy; ° Patients were randomly assigned to receive 360 mg of nivolumab and carboplatin at area under the curve 5 on day 1 every 3 weeks for 4 cycles, plus 80 mg/m2 of paclitaxel on days 1, 8, and 15 every 3 weeks for 4 cycles. This was administered with (n = 53; arm A) or without (n = 55; arm B) a 2-week lead-in of 240 mg of nivolumab. Patients in arm B received the nivolumab lead-in after completing the 4 cycles of chemoimmunotherapy. Abbreviations: 22C3, Dako PD-L1 IHC 22C3 pharmDx; AC, Anthracycline and Cyclophosphamide; CI, confidence interval; Cp, Carboplatin; ddAC, dose-dense Anthracycline and Cyclophosphamide; EFS, Event-Free Survival; ER, Estrogen Receptor; HR, Hazard Ratio; iDFS, invasive Disease-Free Survival; mFUP, median Follow-Up; Nab-P, Nab-Paclitaxel; NA, Not Available; N+, Node-Positive; P, Paclitaxel; pCR, Pathologic Complete Response; PD-1, Programmed Death-1; PD-L1, Programmed Death-Ligand 1; SP-142, Ventana PD-L1 (SP142) Assay; SP263, Ventana PD-L1 (SP263) Assay; TILs, tumor-infiltrating lymphocytes.

supports the hypothesis that administering ICI while the primary tumor is still present may induce a more robust systemic antitumor immune response, likely because more neoantigens are available to activate the immune system [21–24]. The A-BRAVE trial randomized patients with early TNBC who had residual disease (RD) after NACT or were at high risk after upfront surgery and adjuvant CT to receive either adjuvant avelumab or observation. Although the trial did not meet its primary endpoint of disease-free survival (DFS; HR: 0.81; 95% CI, 0.61–1.09, p = 0.172), it showed significant improvement in OS (secondary endpoint; HR: 0.66; 95% CI, 0.45–0.97, p = 0.035) and in distant disease-free survival (DDFS, exploratory endpoint; HR: 0.70; 95% CI, 0.50–0.96, p = 0.0277). While a signal favoring adjuvant immunotherapy was observed in the ~80% of patients with RD after NACT (Stratum B), the benefit for patients who underwent upfront surgery is less certain (Stratum A [adjuvant]: 11/40 events, avelumab arm; 12/43 events control arm), particularly when considering Alexandra data [25]. Ongoing clinical trials such as A012103/OptimICE-pCR and SWOG1418 will further explore ICI optimization in TNBC. These studies are investigating the omission of adjuvant ICI in patients who experience a pCR after NACIT and testing the addition of adjuvant ICI in patients with RD, respectively.

In early ER+HER2- BC, representing 70–80% of all BCs, the mainstay of treatment is represented by adjuvant endocrine treatment (ET) ± cyclin-dependent kinase 4/6 (CDK4/6) inhibitors, with some cases who also benefit from NACT or adjuvant CT [1,5,26,27]. The magnitude of benefit from CT depends on the baseline risk of recurrence (prognostic factors), which may be estimated from clinical features (e.g., stage, grade), as well as biologic features of the tumor (e.g., gene expression) [5] and the intrinsic efficacy of CT (predictive factors) which has been essentially demonstrated for the Recurrence Score [28,29]. A large *meta*-analysis has demonstrated that administration of CT either in the neoadjuvant or in the adjuvant setting is associated with similar outcomes in ER+HER2- BC [30]. Moreover, unlike TNBC and HER2+ BC, lack of pCR does not always translate to worse survival outcomes [2]. Therefore, in early ER+HER2- BC the main goal of administering neoadjuvant instead of adjuvant CT is to improve surgical outcomes.

However, a subgroup of patients with ER+HER2- BC, typically characterized by high-grade disease, luminal B-like genomic features and defined by multigene tests (e.g., OncotypeDX, MammaPrint, Prosigna, EndoPredict) often harbor poor prognosis, decreased sensitivity to adjuvant ET and higher benefit from CT [31,32]. In the phase 2 clinical trial I-SPY2, pembrolizumab added to NACT improved pCR rates from 13.6% to 34.2%, when compared to NACT alone in patients with ER+HER2- BCs with high Mammaprint scores [16,32]. On this basis, two large recently reported RCTs have investigated treatment escalation in predominantly high grade ER+HER2- early BC by adding an ICI to NACT (Table 2) [33]. Both trials demonstrated significant improvement in pCR rates with the addition of an ICI to NACT, but only mature EFS data will clarify whether NACIT will be a treatment option for selected ER+HER2- tumors.

As NACIT has been incorporated into the treatment algorithm for stage II-III TNBC, with potential for expansion into other early-stage

Table 2
Selected phase 2 and 3 clinical trials which investigated the impact of the addition of ICIs to NACT in early ER+/HER2- BC.

Study, Immunotherapy agent (target)	Chemotherapy regimen	Patient population	Subgroup (PD-L1 CPS)	Immunotherapy Number	Immunotherapy pCR (%)	Control arm Number	Control arm pCR (%)	EFS results (exp. arm versus control)
KEYNOTE-756, Pembrolizumab (PD-1) [34]	Paclitaxel q1w for 12 ws, then doxo/epi q3w + cyclophosphamide q2w or q3w for 4 cycles.	All grade 3, ~35% T3-4, ~90% node positive	All	635	24.3	643	15.6	Immature data
			PD-L1+ (CPS ≥ 1 SP142)	482	29.7	489	19.6	
			PD-L1-	153	7.2	154	2.6	
CheckMate7FL, Nivolumab (PD-1) [35]	Paclitaxel q3w for 4 cycles, then doxo- cyclophosphamide q2w or q3w for 4 cycles.	~98% grade 3, ~80% node positive, ~46% stage III	All	257	24.5	253	13.8	Immature data (exploratory endpoint)
			PD-L1+(CPS ≥ 1 SP142)	88	44.3	84	20.2	
			PD-L1-	169	14.2	169	10.7	
I-SPY2.2, datopotomab-deruxtecan and durvalumab [18]	Block B (paclitaxel, carboplatin, pembrolizuman) and/or Block C (AC and pembrolizumab)	Mammaprint high risk, stage II-III, HER2− Immune+ (approximately 1/3 ER+)	NA	47	65.0	NA	NA	Immature data

The first trials evaluating ICIs as monotherapy in patients with ER+HER2- mBC resulted in only modest response rates (e.g., KEYNOTE-028, JAVELIN) [36]. Although CT has historically been considered immunosuppressive [37], robust preclinical and clinical data show that cytotoxic drugs enhance tumor immunity and have synergism with ICIs, especially through CT-induced release of tumor cell NeoAgs from dying cancer cells. In this context, preclinical models have shown that paclitaxel, a tubulin-targeting drug, increases tumor cell permeability to granzyme-B (released from cytotoxic T cells) and upregulated MHC class I expression on cancer cell lines to induce tumor cell immunogenicity [38]. Consistently, taxane-containing chemoimmunotherapy combination have been already approved as first-line treatment in metastatic PD-L1+ TNBC (e.g., Impassion130, only EMA; KEYNOTE-355, FDA and EMA). Given such promising results using chemoimmunotherapy in TNBC, there has been effort to replicate similar strategies in ER+ mBCs. For example, eribulin, a microtubule inhibitor, has been shown to reverse epithelial-mesenchymal-transition, as well as decrease FOXP3 and PD-L1 expression as measured through IHC [39,40]. Eribulin has been investigated with or without pembrolizumab in a phase 2 trial involving 88 patients with pre-treated ER+HER2- mBC [41]. The addition of pembrolizumab to eribulin did not add any benefit and PD-L1 status, TILs and TMB were not associated with median PFS. As 5-FU has been shown to increase the expression of CEA in BC cell lines and to reduce the number of MDSCs in murine models, another phase 2 trial tested the combination of capecitabine, a prodrug of 5-FU, with pembrolizumab in 30 patients with mBC, of whom 14 participants had ER+ ET-resistant disease [42]. Among the 29 evaluable patients, the median PFS was 4 months, the ORR was 14% and the CBR was 28%, without significant differences between TNBC and ER+ BCs. Therefore, given this relatively modest response rate, this regimen was deemed not worthy of further study in BC. While traditional CT did not provide satisfactory results when coupled with ICIs in patients with ER+HER2- mBCs, CT targeted to specific Ags, in the form of ADCs, may serve as a more synergistic drug partner. To test whether ADCs synergize with ICIs, the SACI-IO HR+ trial has investigated whether adding pembrolizumab to sacituzumab govitecan improved PFS compared to sacituzumab govitecan alone in ER+ mBC. The trial was negative and showed a non-significant trend toward improved PFS and OS in PD-L1+ patients (NCT04448886) [43]. An alternate explanation for the lack of ICIs efficacy in ER+HER2- BCs may be the fact that these trials evaluated ICIs in heavily pretreated patients with mBC, which typically show fewer TILs and lower PD-L1 expression as compared with primary BCs [44]. Abbreviations: 5-FU, 5-Fluorouracil; Ags, Antigens; BC, Breast Cancer; CBR, Clinical Benefit Rate; CEA, Carcinoembryonic Antigen; CPS, Combined Positive Score; CT, Chemotherapy; Doxo, Doxorubicin; EFS, Event-Free Survival; EMA, European Medicines Agency; ER, Estrogen Receptor; ER+HER2-, Estrogen Receptor Positive/Human Epidermal Growth Factor Receptor 2 Negative; ET, Endocrine Treatment; FDA, Food and Drug Administration; FOXP3, Forkhead Box P3; ICI, Immune Checkpoint Inhibitor; IHC, Immunohistochemistry; mBC, Metastatic Breast Cancer; MDSCs, Myeloid-Derived Suppressor Cells; MHC, Major Histocompatibility Complex; NACT, Neoadjuvant Chemotherapy; NeoAgs, Neoantigens; ORR, Objective Response Rate; PD-1, Programmed Death-1; PD-L1, Programmed Death-Ligand 1; PFS, Progression-Free Survival; qNw, once N week; TILs, Tumor-Infiltrating Lymphocytes; TMB, Tumor Mutational Burden; TNBC, Triple-Negative Breast Cancer; ws, weeks.

subtypes, research efforts are underway to predict treatment response to ICIs. Indeed, KEYNOTE-522 did not support PD-L1 as a predictor of efficacy of immunotherapy, as benefit from NACIT was observed irrespective of PD-L1 status or other clinical and biologic features. To date, no correlative studies have successfully brought predictive biomarkers for response to NACIT into the clinic. Yet, being able to predict response to NACIT would be useful to provide clinical benefit, while minimizing toxicity. Indeed, treatment-related adverse events (AEs) led to discontinuation of any trial drug in 23.3% of the patients in the pembrolizumab-CT group and 12.3% in the placebo-CT group in KEYNOTE-522 [4]. In a multicenter real-world experience, AEs led to early discontinuation of the KEYNOTE-522 regimen in 39.5% of the 228 participants, with 31.9% experiencing an immune-related AE [45]. Thus, in good responders, even if treated with NACT alone, the risk of immune-related toxicity should be avoided, as it could be severe, irreversible, and potentially impact fertility and quality of life [46]. Conversely, early recognition of poor responders to NACT/NACIT, potentially relapsing soon after surgery, would allow for treatment escalation (e.g., trials for high-risk patients) or for testing alternative treatments or immune combinations [47,48].

This review aims to capitalize on the existing translational and clinical evidence regarding predictors of treatment response in patients with early-stage BC treated with NACT/NACIT. It provides an overview of the complex interplay among ER signaling, cancer cell growth, and the tumor immune microenvironment (TIME). Finally, it sets the stage for discussions on the effects of estrogens and pharmacological ER modulators on immunity and immune cell functions, areas that have been difficult to unify into a coherent model.

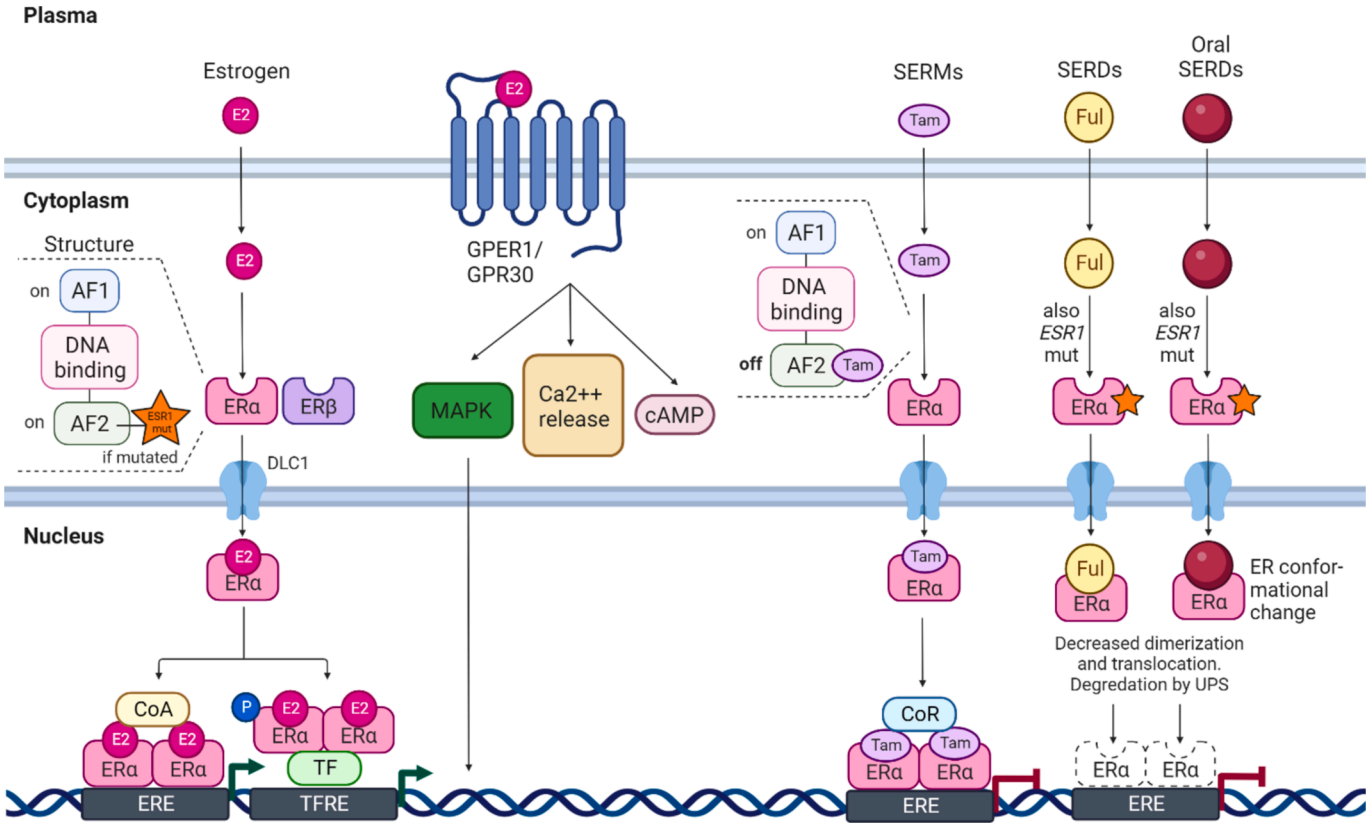


Fig. 1. ER signaling pathway and main mechanisms of action of traditional and novel endocrine agents for breast cancer treatment. Without hormones, the ER resides in the cytoplasm or nucleus of target cells, bound to complexes containing large heat-shock proteins that maintain the receptor in a transcriptionally inactive state. Ligand binding triggers a conformational shift in the receptor, resulting in its separation from inhibitory proteins, dimerization, movement to the nucleus, and interaction with specific enhancers in target genes. The activated ER can directly bind to specific EREs or be tethered to DNA via protein–protein interactions with other transcription factors at relevant enhancers. Once bound to DNA, the receptor can initiate the formation of large complexes comprising various cofactors, whose composition determines the scope and intensity of the transcriptional response. Additional complexity stems from the interaction of signaling pathways, like MAPK, with receptor:coregulator complexes, which can modify the activity of these complexes. In some cases, this alteration enables the ER to activate target gene transcription even in the absence of a classical ligand [49]. In a non-genomic pathway, E2 interacts with GPER1, regulating MAPK, calcium release, and cAMP. SERMs attach to the ER, acting as antagonists in breast cancer cell gene transcription while serving as agonists in other tissues, such as bone, endometrium, and blood vessels. Tamoxifen is the only SERM used in the adjuvant treatment of breast cancer. Among SERDs, fulvestrant was the first to be introduced into clinical practice. It blocks the dimerization and nuclear translocation of the ER, and it facilitates proteasomal degradation. Fulvestrant also retains partial activity in ESR1-mutant ERs, which could otherwise initiate gene transcription independently of a ligand. CERAN and SERCA (both investigational, not shown) ultimately produce the same outcomes as fulvestrant, although they exhibit more potent effects on both wildtype and mutant ERs, with an increased rate of receptor degradation. Additionally, PROTACs (investigational, not shown) consist of one domain that attaches to a target protein and another that links to an E3 ubiquitin-ligase. The proximity of these two components leads to increased vulnerability of the target protein to polyubiquitination and subsequent proteasomal degradation of the ER in cancer cells. Abbreviations: AF1, activation function 1; AF2, activation function 2; Ca, calcium; cAMP, cyclic adenosine monophosphate; CERAN, complete estrogen receptor antagonist; CoA, steroid receptor coactivator; CoR, steroid receptor corepressor; DLC1, deleted in liver cancer 1; E2, estradiol; ERα, estrogen receptor alpha; ERE, estrogen response elements; ESR1, estrogen receptor 1; GPER1, G protein-coupled oestrogen receptor 1; GPR30, G protein-coupled receptor 30; MAPK, mitogen-activated protein kinase; P, phosphorus; PROTAC, proteolysis targeting chimeras; SERCA, selective estrogen receptor covalent antagonists; SERD, selective estrogen receptor degrader; SERM, selective estrogen receptor modulators; Tam, tamoxifen; TF, transcription factor; TFRE, transcription factor response element. Adapted from [53] and created with biorender.com (2024).

ER signaling and its interaction with the immune system

ER pharmacology

Estrogens consist of structurally related steroid hormones that originate from cholesterol. The main estrogens naturally produced include estrone (E1), 17β-estradiol (E2), estriol, estetrol (E4), and 27-hydroxycholesterol (27HC) [49]. Of these, 17β-estradiol (E2) has the highest affinity for the three known ERs. Estrone, synthesized in both the gonads and adipose tissues, is the predominant estrogen in postmenopausal women. In contrast, estriol and estetrol (E4) are produced and circulate at substantial levels only during pregnancy [50]. The physiological function of 27HC remains unclear, though it is believed to display estrogenic activity primarily in postmenopausal women or in younger women when gonadal function ceases [49,51]. The two main nuclear receptors for estrogens, ERα and ERβ, have different roles based on the cells where they are expressed. ERα is more commonly expressed on immune cells. The expression of ERβ is not yet fully understood [52].

From a pharmacodynamic standpoint, the structure of ERα is shaped by the specific characteristics of the ligand it binds to. Even minor modifications to the ligand's structure can lead to substantial changes in ER's surface configuration. Moreover, ER's conformation influences coregulator binding (Fig. 1). This suggests that the ability of different ER-ligand complexes to recruit various coregulators could be the key determinant in their response [49].

In clinical settings, these characteristics can be targeted by Selective Estrogen Receptor Modulators (SERMs), which specifically interact with ERα and exhibit either agonistic or antagonistic effects depending on the tissue. Tamoxifen, often used in the adjuvant treatment of patients with early ER+ BC, is the most frequently prescribed drug in this class.

These drugs can also activate the membrane-bound G-protein-coupled receptor 1 (GPER1, GPR30). When engaged, this receptor allows estrogens to trigger non-genomic effects (Fig. 1) [49,54]. Finally, aromatase (CYP19) inhibitors (AIs) quantitatively block the conversion of androgens into estrogens, impacting various cells differently. In reaction to the estrogen depletion caused by these inhibitors, some cells may boost the expression of coregulators, facilitating ligand-independent activation of the ER.

Sex hormones modulate the immune system

The biological differences between men and women influence the development and functioning of the immune system [55]. The interaction between sex and immunity is complex and multi-dimensional. Females generally show greater resistance than males to a broad range of pathogens [55,56]. However, females are also more prone to most autoimmune diseases [55]. Differences in the ability to mount innate and adaptive immune responses contribute to differing levels of disease susceptibility, including cancer [55,56].

In general, females exhibits a stronger response of phagocytes to toll-like receptor (TLR) stimuli. Differences have been highlighted in T cell polarization, with males and females tending to develop biased Th1 and Th2 responses, respectively. Of note, the production of interferons by macrophages and dendritic cells (DCs), typically linked to type 1 immunity, has been observed to be higher in females than in males [56]. A preferential Th17 skewing has been observed in males. Finally, consistent data suggest that females exhibit more effective B cell responses to various antigens, including a number of vaccines [56]. There does not appear to be a significant difference in the prevalence or risk of death from sepsis between sexes, although the data remain fragmented [57,58]. Summarized below is a selected overview of the interaction between various immune cell types and the ER pathway. Most of the available evidence comes from preclinical models and historical data.

The role of estrogen signaling in innate immunity

Macrophages. Phagocytosis and degranulation of macrophages and neutrophils are generally more efficient in females than in males [55]. In this context, estrogens are known to influence the recruitment, differentiation, polarization, and functionality of macrophages (Fig. 2).

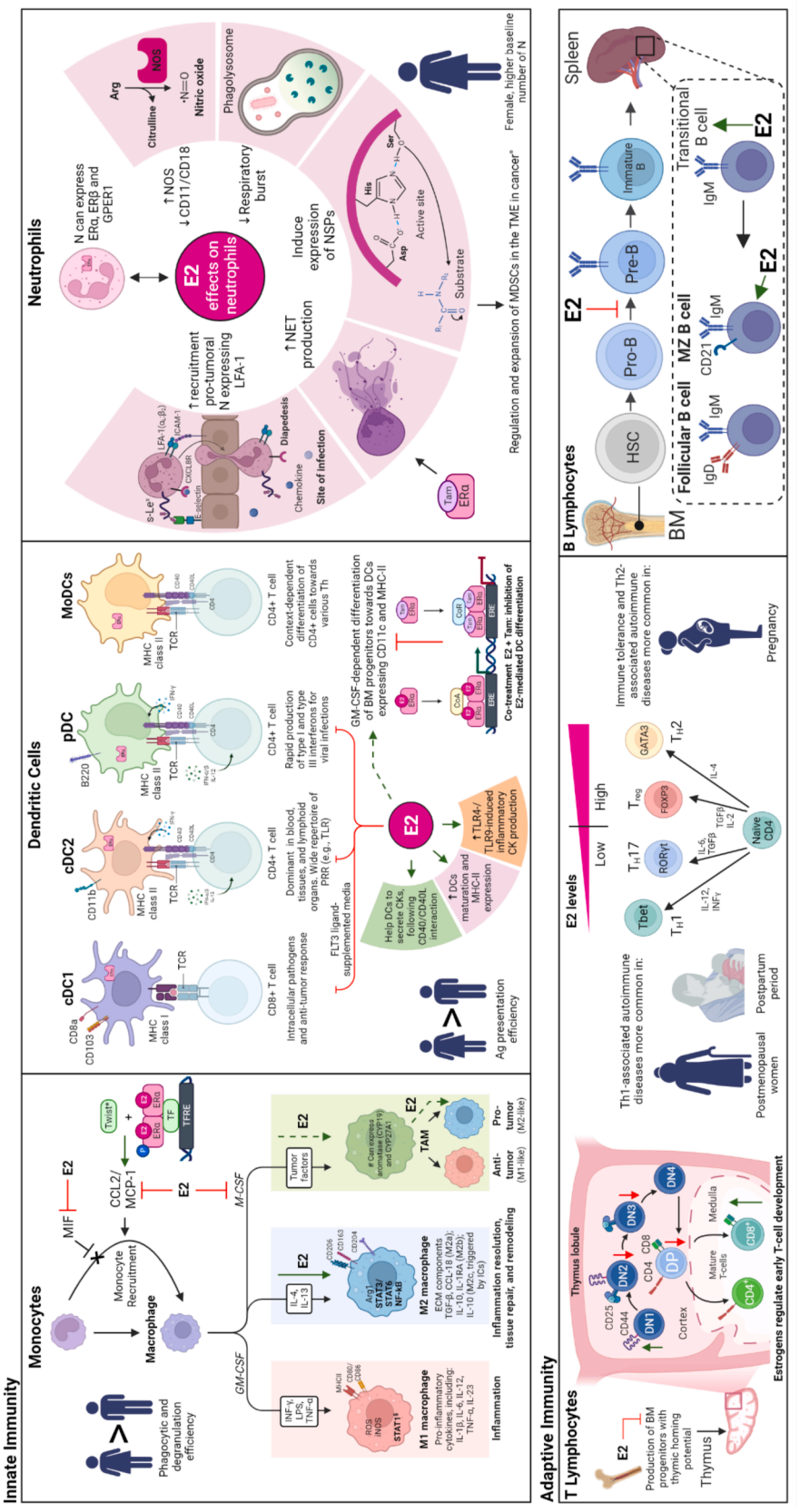
Furthermore, estrogens have been shown to suppress the expression of monocyte colony-stimulating factor (MCSF), potentially impacting the recruitment of monocytes to secondary organs or tumors [49]. Estrogens also polarize macrophages to an M2-like phenotype [49].

In many cancers, tumor-associated macrophages (TAMs) represent the largest myeloid population within the TIME and often, though not always, their abundance is associated with a poor prognosis [61]. TAMs contribute to tumor progression not only through their immunosuppressive functions, but also by secreting vascular endothelial growth factor (VEGF) to enhance angiogenesis and matrix metalloproteinases that facilitate extracellular matrix remodeling. Finally, TAMs can express both aromatase and CYP27A1, allowing them to produce E1, E2, and 27HC in situ, which influences their ER biology (Fig. 2) [62].

Dendritic cells. All DCs subsets (conventional DCs [cDCs], plasmacytoid DCs [pDCs], and monocyte-derived DCs [MoDCs], Fig. 2) express ERα, and they typically present antigens to T cells more effectively in females than in males [55,63]. In this context, E2 has been found to suppress the development of both pDCs and cDCs in FLT3 ligand-enhanced media, while it promotes GM-CSF-mediated differentiation of bone marrow (BM) progenitor cells into DCs that exhibit the lineage markers CD11c and major histocompatibility complex (MHC)-II [64]. Moreover, estrogens can impact DCs by altering TLR signaling [49]. For instance, E2 promotes TLR4- and TLR9-induced inflammatory cytokine production; DC-intrinsic ERα signaling increases MHC-II and CD86 expression on cDCs [65]. Notably, ERα-deficient DCs retain the capacity to produce inflammatory cytokines like IL6 and TNFα upon stimulation through TLR3/4. However, they do not secrete these cytokines following CD40-CD40L interactions [65]. Finally, E2 enhances maturation of DCs and stimulates MHC-II expression in these cells [49,63]. While enhancing DC function via E2 could be a key target for developing adjuvant drugs in cancer immunotherapy, the signaling and pharmacology of E2 in dendritic cells within tumors are shaped by various tumor-derived factors and other cells in the TIME. This complex interaction is an active area of research.

Neutrophils. Neutrophils express both ERα and ERβ, and the levels of these receptors in these cells differ by sex and menopausal status [66,67]. On average, females exhibit a higher baseline number of neutrophils compared to males, a variance influenced by fluctuations in serum E2 levels that occur during the reproductive cycle and pregnancy [68,69]. The ability of E2 to induce the expression of neutrophil serine proteases is a key mechanism by which this hormone activates neutrophil-dependent inflammatory responses (Fig. 2) [70]. Activation of the ER has also been demonstrated to enhance the expression and activity of neuronal nitric oxide synthase (NOS) and reduce the expression of the cell adhesion proteins CD11/CD18 in human neutrophils (Fig. 2) [49]. However, the most well documented function of neutrophils related to ER activity is their ability to produce and release neutrophil extracellular traps (NETs), which physically entrap pathogens (Fig. 2) [71]. The relationship between E2 and neutrophils becomes more complex considering the seemingly paradoxical observation that E2 can inhibit the activation of the respiratory burst induced by pathogens in these cells (Fig. 2) [72]. Furthermore, in BC cell lines, E2 enhances the recruitment of pro-tumoral neutrophils that express lymphocyte function-associated antigen-1 (LFA-1), an integrin that facilitates cancer metastasis, to the invasive front of tumors [73]. In the same BC models, treatment with fulvestrant (Fig. 1) was linked to reduced accumulation of neutrophils and macrophages in the TIME, along with a decrease in pro-angiogenic and immune-suppressive cytokines [74].

Myeloid-derived suppressor cells (MDSC). The mobilization and proliferation of granulocytic-MDSCs (gMDSCs) and monocytic MDSCs (mMDSCs) during pregnancy, in autoimmune diseases, and within the TIME are influenced by E2 [49]. For instance, at the maternal-fetal



(caption on next page)

Fig. 2. Representation of the intricate and not entirely understood interaction between the ER signaling pathway and specific cell types from both the innate and adaptive immune responses. Most evidence originates from preclinical data, with details about the system model used to gather this evidence provided. *In ER+ BC, E2/ER enhances the production of CCL2 from tumors through a cooperative interaction with the transcription factor Twist. This increase in CCL2 subsequently promotes cancer cell proliferation, invasion, and metastasis in validated xenograft models of the disease [59]. § Evidence indicates that E2 can positively influence M1 polarization by modulating STAT1. Specifically, E2 boosts the proteolytic cleavage and activation of STAT1, enabling DNA binding and triggering the expression of genes linked to inflammatory responses. This may be relevant in hepatocellular carcinoma, where morbidity and mortality rates are higher in men compared to women [59]. In this condition, estrogens offer protection by inhibiting the alternative M2 activation of TAMs, thereby shifting them towards an M1-like phenotype. Although it might seem counterintuitive for E2 to positively affect both M1 and M2 polarization, recent insights into ER pharmacology have shed light on how this occurs through the selective use of coregulators [59]. # TAMs can express both CYP19 (aromatase) and CYP27A1, and therefore can produce E1, E2, and 27HC in situ [59]. ◦ In preclinical models of myeloproliferative diseases that originate from immature progenitor cells like MDSCs, tamoxifen has been effective in inhibiting the expansion of these progenitors, suggesting a possible utility for this subclass of ER modulators [55,60]. Abbreviations: Arg, arginine; Asp, aspartic acid; BM, bone marrow; CCL2, chemokine (C–C motif) ligand 2; CD, cluster of differentiation; cDC, conventional dendritic cell; CXCR, C-X-C chemokine receptor; DN, double negative; DP, double positive; E2, estradiol; ER, estrogen receptor; FLT3, FMS-like tyrosine kinase 3; FOXP3, forkhead box P3; His, histidine; HSC, hematopoietic stem cell; ICAM, intercellular adhesion molecule; IL, interleukin; IL-R, interleukin receptor; Ig, immune globulin; INF, interferon; LFA-1, lymphocyte function associated antigen 1; LPS, lipopolysaccharides; M−CSF, macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; MDSC, myeloid-derived suppressor cell; MHC, major histocompatibility complex; MIF, Macrophage migration inhibitory factor; MoDC, monocyte-derived dendritic cell; MZ, marginal zone; N, neutrophil; NET, Neutrophil extracellular trap; NF-kB, nuclear factor kappa B; NOS, nitric oxide synthase; NSP, neutrophil serine proteases; P, phosphate; pDC, plasmacytoid dendritic cell; RORγt, RAR-related orphan nuclear receptor γt; Ser, serine; sLeX, Sialyl LewisX (CD15s); TAM, tumor-associated macrophage; Tam, tamoxifen; Tbet, T-box transcription factor TBX21; TCR, T-cell receptor; TF, transcription factor; TFRE, transcription factor regulatory element; Th, T helper; TME, tumor microenvironment; TNF, tumor necrosis factor. Created with biorender.com (2024).

interface, MDSCs inhibit T-cell function and activation to prevent fetal rejection [75]. In tumors, MDSCs can be predominant in the TIME, where they actively suppress anti-tumor immunity and also secrete factors that contribute to tumor growth and metastasis [49]. In this scenario, E2 not only reduces T-cell anti-cancer activity by stimulating MDSCs, but also directly promotes tumor growth through ER-dependent activation of the JAK/STAT pathway, which is crucial for the expansion of MDSCs [76,77]. As a result, preclinical studies support the use of tamoxifen as an adjuvant in immunotherapy due to its ability to negatively regulate MDSC function within the TIME [60].

The role of estrogen signaling in adaptive immunity

T lymphocytes. Estrogens impact T-cell biology across their entire lifecycle, from maturation through the modulation of effector functions [49]. For instance, estrogens influence the development of T cells in the thymus (Fig. 2). First, progenitor cells lacking lineage markers migrate from the BM to the thymus, where they mature into CD8+ and CD4+ T-cells. Estrogens decrease the number of these progenitors that can home to the thymus. Second, within the thymus, T cells progress through various stages of selection. Exposure to estrogens results in a reduction of the double negative (DN)2, DN3, and double positive (DP) populations, while increasing the double negative (DN1) and single positive CD8+ and CD4+ populations [49]. Additionally, estrogens have diverse impacts on T helper cell differentiation (Fig. 2). CD4+ T cells differentiate into various T helper cell subclasses in response to distinct environmental signals, including estrogens. Th1 and Th2 cells reciprocally influence each other's differentiation, similar to the interaction between Th17 and Treg cells. Higher estrogen levels encourage the transformation of naïve T-cells into Th2 and Treg cells, respectively. The importance of estrogens in T cell development and function is highlighted by the differences in the prevalence and severity of autoimmune diseases between males and females [49]. Women have a higher incidence of rheumatoid arthritis, multiple sclerosis, and Crohn's disease. Of note, E2 exerts a dual effect on the pathology of T cell-mediated autoimmune diseases (Fig. 2). Th1-associated autoimmune diseases are more common in postmenopausal women or immediately postpartum. On the other hand, Th2-associated autoimmune diseases often exacerbate during pregnancy. Finally, evidence mainly derived from autoimmune diseases suggests that estrogens can directly modulate the PD-1/PD-L1 pathway [55].

B lymphocytes. Females typically have higher B cell counts than males, resulting in elevated basal antibody levels and stronger responses to several vaccines [55]. E2 role in regulating early B-cell development is multifaceted. During the early stages of differentiation, E2 inhibits the transition of pre-B cells to pro-B cells in the BM. As B-cell development advances, however, estrogens promote the differentiation of transitional B cells, marginal zone B cells, and the generation of autoantibodies from memory B cells (Fig. 2). In this phase, SERMs can disrupt B-cell development, particularly during the transition from pre-B cells to immature B cells. Notably, SERMs do not influence transitional, marginal zone, or follicular B cells [49].

Sex hormones affect the anticancer immune response and ICI efficacy

Differences in the molecular mechanisms of spontaneous (i.e., non-drug-induced) anticancer immune responses have been acknowledged between females and males across various tumor types [55]. Consistently, a significant sex-based heterogeneity of ICI efficacy has been documented across different solid tumors [78,79]. A recent *meta*-analysis of RCTs examining ICIs monotherapy versus conventional treatments not involving immunotherapy showed a greater survival benefit for males compared to females. This gender gap remained consistent across different cancer types, lines of treatment, and the specific type of ICI administered [78]. By using a predictive machine-learning approach, a study examining clinical and multidimensional molecular data from more than 1,000 patients diagnosed with advanced solid tumors and treated with ICIs revealed a comparable trend: male sex was significantly and independently associated with a greater treatment response [79]. The toxicity profile of ICIs has also been demonstrated to be influenced by sex [55]. A systematic review of all the AEs reported in prospective trials conducted by the SWOG Cancer Research Network, involving 2,319 patients with solid and hematological tumors and testing various immunotherapy regimens, found that females had a 49% higher risk of experiencing severe toxicity compared to males [80]. Interestingly, the tolerability profile of ICIs in females appeared to be influenced by menopausal status: the risk of developing immune-related AEs was significantly higher in premenopausal females than both postmenopausal females and males [55,80]. Within this framework, the subsequent paragraphs describe the main molecular mechanisms that underlie the differences in the anticancer immune response and the efficacy of ICIs based on sex, particularly focusing on the immune assets specific to females.

Impact of sex hormones on the innate anticancer immune response

Evidence primarily from preclinical models indicates that the sex-based differences in antitumor immunity can also be influenced by the innate immune system [81]. Changes in the prevalence of specific subsets of MDSCs and their distribution within tissues have been observed in mice with glioblastoma and are linked to survival outcomes: male mice exhibit increased accumulation of mMDSCs in the TIME, whereas

female mice show higher counts of gMDSCs in the peripheral blood [81]. Consistently, estrogens have been associated with the recruitment of MDSCs in liver metastases across various tumor types such as colorectal, lung, and pancreatic carcinoma, thereby contributing to an immunosuppressive TIME [82]. Furthermore, the addition of E2 accelerated tumor growth in immunocompetent mouse recipients when various melanoma cell lines were transplanted, but not in immunocompromised mice, indicating an effect influenced by immune cells beyond the tumor [55]. This phenomenon was found to be due to ER-mediated polarization of TAMs toward the immune-suppressive M2-like phenotype [49]. Notably, inhibition of ERα through fulvestrant decreased tumor growth, stimulated adaptive immunity, and increased the antitumor efficacy of ICIs [49].

Impact of sex hormones on the adaptive anticancer immune response

Data from The Cancer Genome Atlas (TCGA) showed that tumors in females may undergo a more intense immunoediting process during the early stages of tumor progression [83]. Compared to tumors in males, those in females are more likely to accumulate mutations that are not effectively presented by their specific MHC-I and MHC-II molecules. This phenomenon likely results from a more robust immune-mediated elimination of cancer cells that carry mutations well-presented by these molecules [83]. This immunoediting process seems to be more pronounced in younger females and primarily affects mutations presented by MHC-II molecules, consistent with higher CD4+ T cell counts found in females compared to males [55,83,84]. For example, the TIME of females with early-stage non-small cell lung cancer (NSCLC) is significantly enriched with DCs, CD4+ T cells, and B cells, and shows greater clonality of tumor-infiltrating lymphocytes (TILs) compared to males [85]. Despite better immune recognition and response, NSCLCs in females evolve more elaborate and redundant resistance mechanisms, evidenced by a higher exhaustion status of intratumoral CD4+ and CD8+ T cells [55]. Specifically, the TIME in female patients features increased expression of several immune checkpoint molecules with inhibitory properties, including T-cell immunoglobulin mucin 3 (TIM3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), and V-domain Ig suppressor of T cell activation (VISTA). Additionally, there is a greater presence of immune-suppressive cells such as cancer-associated fibroblasts (CAFs) and MDSCs [85]. Similar observations have been made in patients with melanoma and glioblastoma, both of which are cancers known to display sex-based disparities in survival outcomes [55,86].

The tumor-immune ecosystem of TNBC

High genomic instability and mutational burden in TNBC lead to an increased likelihood of producing neoantigens, which the adaptive immune system can identify as 'non-self' [24]. In patients with early-stage TNBC, the prognostic and predictive importance of TILs and immune-related gene expression signatures is well recognized [87–89]. A recent pooled analysis of two multi-site prospective studies, involving 474 patients with stage I (T > 1 cm)–III TNBC treated with anthracycline-free NACT, showed that the density of stromal TILs independently stratified long-term outcomes, providing prognostic insights beyond those offered by clinical-pathological variables and pathological response [90]. TILs were shown to significantly refine outcome predictions in patients who experienced a pCR, especially among those with stage II-III disease [90]. Similar findings were observed in a smaller multi-site cohort (n = 223) of patients who experienced a pCR following NACT [91]. In a retrospective study including 1966 patients with early-stage TNBC treated only with locoregional therapy, those with higher levels of TILs had significantly improved survival rates. Specifically, patients with a TIL level of ≥ 50% had a 5-year survival rate of 90%, compared to 72% for those with less than 30% TILs [92].

The composition and spatial arrangement of these infiltrates have also become recognized as relevant factors [24,93]. Tumors with uniformly low or high numbers of CD8+ T cells are categorized as immune desert and fully inflamed, respectively. Tumors where CD8+ T cells are confined to the tumor margins or stroma are described as margin-restricted and stroma-restricted, respectively, both featuring few or no intratumoral lymphocytes. Immune desert, or 'cold' tumors, have scant immune cell infiltrates primarily composed of PD-L1+ TAMs and exhausted T cells. In compartmentalized tumors, where immune cells are spatially separated from tumor cells, neutrophils are concentrated just outside the tumor border, B cells are positioned further away, and indoleamine 2,3-dioxygenase (IDO1) and PD-L1 are mostly expressed on immune cells, with PD-1 specifically expressed on CD4+ T cells [94,95].

Single-cell RNA sequencing provides detailed insight into the TIME. For example, a single macrophage may express genes linked to both M1 and M2 phenotypes simultaneously, challenging the traditional mutually exclusive polarization model [96]. T cells also show a spectrum of activation and differentiation states and CD8+ T cells with a tissue-resident memory-like phenotype have been identified as key players in the immune surveillance of TNBC. A gene signature associated with these cells correlates with improved DFS and OS in patients with TNBC [97,98].

Considering the TNBC TIME as an ecosystem, high levels of immune infiltration are linked with decreased clonal heterogeneity, fewer somatic copy number alterations, and a reduced load of somatic mutations and neoantigens. This suggests that immune surveillance might consistently remove immunogenic clones [99]. Conversely, aneuploidy has been linked to poor immune cell infiltration and immune evasion across various tumor types [100]. Furthermore, specific genomic alterations, such as *TP53* mutations, have been shown to suppress innate immune signaling and promote tumor immune evasion [101,102]. Similarly, the heterozygous deletion of 17p, the location of *TP53* which is found in 41.9% of TNBC, is linked with poor prognosis and is notably associated with decreased immune infiltration by cytotoxic T cells [103]. Additionally, DNA damage response deficiency may alter the TIME by activating the cGAS–STING pathway, and evidence of DNA immune response signatures has also been identified [104,105]. Finally, external factors from cancer, such as interferons and estrogens as previously discussed, can alter PD-L1 expression. Moreover, cancer cell-related mechanisms, including gains and amplification of *CD274* (the gene encoding PD-L1), and the interaction of nucleophosmin with the CD274 promoter in TNBC cell lines, are associated to increased PD-L1 expression in BC cells [106–108].

Within the context of a dynamic co-evolution between tumor cells and the TIME, from early stages to metastatic disease, TNBC progressively shows increased tumor mutational burden, clonal diversity, large-scale chromosomal alterations, and more frequent biallelic loss-of-function mutations in genes linked to homologous recombination DNA repair [109–112]. Conversely, the expression of genes involved in different parts of the antigen-processing machinery, including MHC class I, the antigen peptide transporter complex (like transporter associated with antigen processing 1, TAP1), and the immune proteasome, is reduced in metastatic TNBC [44]. Metastatic sites show a higher degree of immunodepletion, marked by a fewer TILs, CD8+ T cells, and DCs. There is also reduced expression of immunomodulatory genes and signatures, including those related to interferon and *CD274* [44,110]. Conversely, metastases display an increased presence of macrophages and sustained expression of genes linked to various immune evasion mechanisms, such as human leukocyte antigen (HLA)-G, T-cell immunoglobulin and mucin domain 3 (TIM3), lymphocyte-associated gene 3 (LAG3), CD73, chemokine (C–C motif) ligand 2 (CCL2), C–C chemokine receptor type 2 (CCR2), and colony stimulating factor 1 receptor (CSF1R) [44]. Differences between metastatic sites have also been described [113].

The tumor-immune ecosystem of ER+HER2- BC

Typically, ER+HER2- BC is associated with low levels of TILs and PD-L1, and, overall, it is less immunogenic than TNBC [41,114,115]. A *meta*-analysis of six randomized trials by the German Breast Group indicated that high baseline levels of TILs predicted a better pathological response to NACT across all BC subtypes. However, while a 10% increase in TILs correlated with longer DSF in TNBC and HER2+ BC, this association was not observed in luminal HER2- tumors. Notably, an increase in TILs was linked to shorter survival in luminal HER2- tumors [116]. Assessment of TILs in ER+HER2- primary BC before and after NACT demonstrated that low TIL levels post-NACT, but not at baseline, were significantly associated with improved recurrence-free survival [117]. In highly proliferative (Ki67-high) BCs, high TILs were associated with favorable DFS across all subtypes [118]. Conversely, in the ER+HER2- subtype, increasing TIL levels were associated with poorer DFS in cancers with low Ki67 expression (defined as $\leq$ 25%) [118]. In young women (age $\leq$ 40) with early ER+HER2- BC, high stromal expression of T helper cells (CD3+ CD8-) was associated with better invasive breast cancer-free survival (iBCFS) and distant disease-free survival, even after adjusting for T/N categories, grade, and chemotherapy receipt. High stromal expression of CD3+ CD8- and FOXP3+ CD3+ TILs was associated with better OS and iBCFS in adjusted analyses only [119]. Regarding NACIT, an enriched benefit from ICI on pCR and residual cancer burden (RCB) 0–1 rates was observed in patients with high-risk ER+ tumors exhibiting sTIL $\geq$ 5% in CheckMate7FL [120].

These findings underscore the complexity of the interaction between cancer cells and the TIME. Indeed, for anti-tumor T cell responses to occur, tumor antigens need to be presented in conjunction with HLA molecules [121]. In a study using immunohistochemistry (IHC) to evaluate the frequency of HLA class I downregulation across various cancer tissues (n = 246), HLA-I was either lost or diminished in 85% of BCs [114,122,123]. Other studies have reported HLA-I downregulation in 32.5% of BCs, with a significant association with high-risk clinical features, such as nodal involvement and advanced stage, as well as with a shorter disease-free interval [124,125]. Among 863 patients with BC enrolled in the GeparTrio clinical trial whose HLA-I expression was analyzed, those with ER+HER2- BC exhibited the lowest levels of HLA class I expression compared to other subtypes [126]. A negative correlation between mRNA expression of the estrogen receptor 1 (*ESR1*) gene and HLA was identified in the Cancer Cell Line Encyclopedia, while TCGA also found *ESR1* expression to inversely correlate with *HLA-A* and *CD8B* gene expression [127]. These studies suggest that HLA expression might be inversely related to ER expression and positively associated with T cell infiltration, although the mechanisms behind this relationship have not yet been fully elucidated [128].

Differences in the expression of antigen-processing machinery (APM) have been observed between primary BCs and matched brain metastases in a study involving 65 patients, 49 of whom were ER+ [129]. Primary BC lesions from patients who subsequently developed brain metastases exhibited reduced expression of beta 2 microglobulin (B2M; the co-receptor for HLA) and other components of the APM, including TAP1/2 and calnexin [129]. CD8+ T cell infiltration was significantly higher in primary BC lesions without associated brain metastases and correlated with TAP1 expression [129]. Preclinical data further corroborate these observations. Murine tumor cells transfected with silencing hairpin RNA (shRNA) for TAP1 showed reduced susceptibility to cytotoxic T lymphocytes in vitro and an increased incidence of spontaneous brain metastases in vivo [129].

While reduced expression of MHC-I may hinder CD8+ T cell recognition and response in ER+ BCs, the lack of MHC-I molecules makes ER+ cell lines highly vulnerable to NK cell cytotoxicity [114,130]. Additional factors include pro-tumorigenic TAMs, which have been shown to induce endocrine resistance in ER+HER2- BC cells both in vitro and in vivo via NF-kB and IL-6-dependent signaling pathways [114,131–133]. Indeed, while a higher proportion of M1-like TAMs (Fig. 1) in ER+ BCs is associated with increased rates of pCR and longer DFS and OS, the expression of M2-like TAM-specific genes is elevated in residual BC disease following NACT [134]. Consistently, a retrospective study involving 96 patients with early-stage ER+HER2- BC treated with NACT showed that high levels of tissue-based and gene expression-based lymphocyte and macrophage markers were associated with favorable pathological responses post-NACT [115]. Additionally, paired pre- and post-NACT samples documented a significant decrease in TILs and CD8+ cells following treatment, accompanied by an increase in various myeloid signatures. Specifically, single gene expression analysis revealed increased expression of genes associated with immunosuppressive (M2-like) macrophages in RD post-NACT [115]. Consistent with these results, a supervised analysis of microarray data from the ACOSOG Z1031 trial identified that targetable immune checkpoints such as IDO1, LAG3, and PD-1 were linked to treatment resistance in luminal B BCs. In tissue microarrays, IDO1 was found in stromal cells and TAMs, occurring more frequently in luminal B cases, and showed a statistically significant correlation with STAT1 at the protein level [31]. Expression of the IFN-γ/STAT1 pathway components was linked to higher baseline Ki67, reduced ER and PR mRNA levels, and poorer disease-specific survival [31].

The evidence outlined above supports the notion that estrogens can influence immune cells [135]. For instance, anti-estrogen therapy has been found to increase the expression of α-lactalbumin, a lactation protein inhibited by E2 and a recognized target for vaccination in TNBC, on BC cells [136–139]. In preclinical research, SERD enhanced immune-stimulatory activity by suppressing myeloid cells and, when used alongside anti-PD-L1 therapy, promoted tumor regression and activated anti-cancer TAMs and T cells [139]. Anti-estrogen therapy has also been demonstrated to regulate the expression of CD47, a widely expressed cell-surface receptor that blocks phagocytosis signaling by interacting with SIRP1a on macrophages ("don't eat me" signal) [140]. High levels of CD47 expression are associated with poorer survival in ER+HER2+ BC, but not in TNBC [140]. Elevated CD47 expression in tumors resistant to ET suggests a role for CD47 in mediating anti-estrogen resistance [141]. Such findings may offer therapeutic utility in the treatment of ER+ BCs, especially those resistant to ET (NCT03393845) [114].

Combining current knowledge to predict neoadjuvant treatment response

The evidence discussed thus far lays the groundwork for exploring the factors that influence BC responsiveness to NACT and NACIT. BC has pioneered prognostic and predictive genomic assays [28,29,142]. These tests yield outputs that influence shared patient-physician decision-making, particularly regarding the potential added benefit of adjuvant CT for patients with early ER+HER2- BC. Currently, no assays are available to predict therapy response (NACT/NACIT) in TNBC or forecast immunotherapy response in BC [143].

A recent study gathered multi-modal data from pre-treatment BC biopsies from 168 patients undergoing NACT, with or without HER2-targeted treatment [144]. The extent of RD in the surgical specimens was consistently linked to pre-treatment characteristics, encompassing tumor mutational and copy number profiles, immune cell infiltration, T cell dysfunction and tumor proliferation. In an external validation group of 75 patients, a machine learning approach using such multi-omic data accurately predicted pCR with an area under the curve (AUC) of 0.87 [144]. In this context, while it was previously known that genomic signatures linked rapidly proliferating BCs to a higher likelihood of responding to NACT [145], recent findings have highlighted the role of quiescent cancer cells (QCCs) in contributing to resistance against various cancer treatments (Fig. 3) [146,147].

Specifically, QCCs have been identified in premalignant breast lesions (such as ductal carcinoma in situ, DCIS), and specific protein markers have been established for their identification (e.g., $AKT1^{low}$) [149]. Consistently, novel methods like a Multivariate Proliferation

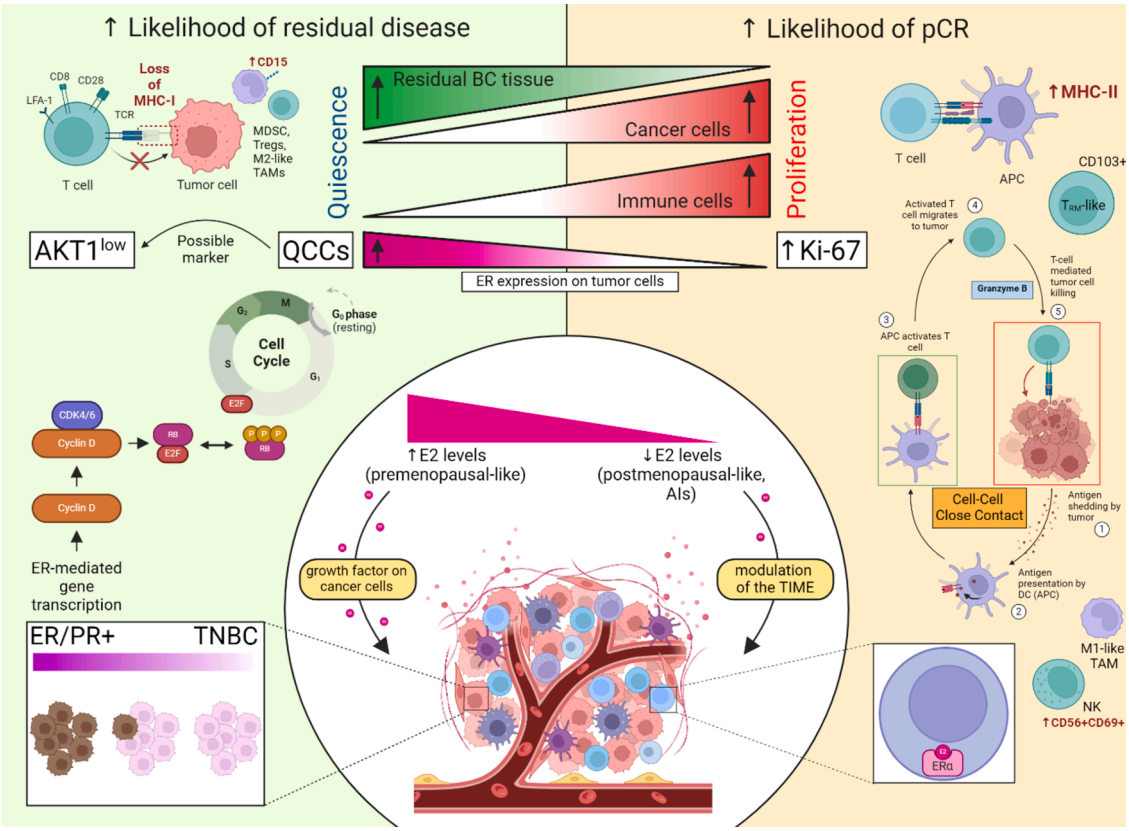


Fig. 3. Proposed holistic view of the interaction ecosystem among the TIME, cancer cells, circulating estrogen levels, tumor intracellular ER-dependent signaling, proliferation versus quiescence, and selected immune cells. Each element is positioned based on evidence suggesting an increased or decreased likelihood of achieving a pCR. Most evidence regarding immune cells pertains to TNBC, with detailed evidence further described in the text. Abbreviations: pCR, pathologic complete response; CD, cluster of differentiation; MHC, major histocompatibility complex; LFA-1, lymphocyte function-associated antigen 1; TCR, T cell receptor; MDSC, myeloid-derived suppressor cells; TAM, tumor-associated macrophages; AKT, protein kinase B; QCC, quiescent cancer cell; APC, antigen-presenting cell; E2, estradiol; CDK4-6, cyclin-dependent kinases 4 and 6; ER, estrogen receptor; PR, progesterone receptor; TNBC, triple-negative breast cancer; TIME, tumor immune microenvironment; NK, natural killer cells. [55,93,97,144,146,148].

Index (MPI), utilizing Cyclic Immunofluorescence (CyCIF), are in development aiming to precisely track proliferating cells and QCCs in both patient and model tissues [148,150].

Regarding NACIT, the 70-gene signature MammaPrint (MP) classifies patients with early-stage BC as having UltraLow, Low, High 1 (H1), or High 2 (H2) risk of distant recurrence. The I-SPY 2 trial showed that patients with MP H2, ER+HER2- tumors have significantly higher response rates to NACIT compared to patients with MP H1 tumors. An in-silico analysis of full transcriptome data from 2916 patients with ER+HER2- MP High Risk BCs showed that H2 tumors had a significantly higher frequency of antigen-presenting cells, including activated dendritic cells and macrophages, CD4+ memory T cells, CD8+ T cells, memory B cells, and plasma cells, compared to H1 tumors. Genes expressing PD-1 and PD-L1 and genes involved in antigen processing, including B2M and TAP1/2, as well as presentation (MHC class I: HLA-A, −B, −F; class II: HLA-DM, −DQ), were significantly upregulated in H2 compared to H1 [151]. The 53-gene signature ImPrint was evaluated in 5 immunotherapy-based arms involving 343 patients with early-stage HER2- BC in I-SPY2 [152]. A larger number of immune markers predicted response to immunotherapy in ER+ than in TNBC, with 27 out of 32 biomarkers being predictive in immunotherapy-based combination arms for ER+. Tumor-immune signatures dominated by chemokines and cytokines were most consistently associated with pCR across immunotherapy arms and receptor status [152]. These markers also correlate with spatial co-localization of PD1+ immune and PD-L1+ tumor cells, especially in TNBC. In ER+ BCs, 28% of cases were ImPrint+ with pCR rates of 76% in ImPrint+ versus 16% in ImPrint-. In TNBC, 46% were ImPrint+ with pCR rates of 75% in ImPrint+ versus 37% in ImPrint- [152]. Finally, across 7 patient cohorts (n = 2304), a B-cell/immunoglobulin signature (IGG) was associated with improved EFS and OS. IGG also predicted pCR in CALGB-40603 and BrighTNess trials [153]. Whether any of these signatures will make it to clinical practice remains to be clarified, as further prospective validation of their prognostic and predictive value is awaited.

Moreover, both foundational principles in immunology and the accumulating evidence focused on the anti-cancer immune response suggest that cell–cell interactions are required within the TIME for NACIT response [93]. Therefore, the impact of multicellular spatial organization on treatment response is under study. Recently, imaging mass cytometry has been employed to generate high-plex tissue images to analyze the expression of 43 proteins at a subcellular level in tumor samples obtained from patients with early TNBC enrolled in the Neo-TRIP RCT [93]. This trial compared NACT (carboplatin and nab-paclitaxel) with NACIT (carboplatin, nab-paclitaxel, and atezolizumab) [9]. Formalin-fixed paraffin-embedded (FFPE) tumor samples were collected at three different timepoints: pre-treatment (baseline), on-treatment (on the first day of the second treatment cycle) and post-treatment (at surgical excision of the tumor bed) [93]. Multivariate modelling showed that the fractions of proliferating (i.e., Ki67-High) CD8+/transcription factor T cell factor 1 (TCF1)+ T cells and MHC-II+ cancer cells were dominant predictors of atezolizumab response (pCR), followed by cancer–immune interactions with B cells and granzyme B+ T cells. An exploratory analysis confirmed that a high density of CD8+TCF1+Ki67+ was linked to increased EFS solely in the case of atezolizumab addition [10]. On-treatment, immune-responsive tumors contained abundant granzyme B+ T cells, whereas resistant tumors were

characterized by CD15+ cells (3-fucosyl-N-acetyl-lactosamine; its sialyl derivative, CD15s) [93]. The same RCT served as a platform for evaluating a 27-gene immune signature, derived from the findings of TNBCtype, an unsupervised k-means cluster classification of TNBC [154–156]. Among patients with a positive signature (referred to as IO+; n = 30), there was a 69.8% rate of pCR following NACIT, compared to a pCR rate of 46.9% for IO- (n = 23) patients [157,158]. Particularly among PD-L1-negative tumors, the difference in pCR rates was more striking (pCR: IO+, 75%; IO-, 31%). Similarly, in the phase II NeoPACT clinical trial, the IO score displayed high prognostic value (pCR: IO+, 81%; IO-, 43%) [13].

Conclusions and future directions

There is a growing body of evidence indicating that the effectiveness of NACT and NACIT may correlate with pre-treatment tumor characteristics, including mutational profiles, ER expression and signaling, immune cell presence and spatial organization, specific gene signatures, and the levels of rapidly proliferating cells versus QCCs. However, the challenge remains that each study often focuses on a specific characteristic in a descriptive manner, and there is still difficulty in synthesizing all the available knowledge into a comprehensive, holistic, and intersectional framework. The predominantly qualitative and descriptive nature of the studies reviewed underscores the current lack of quantitative, reproducible methods that integrate all the composite potential response determinants into a validated, comprehensive, and multimodal predictive assay.

Despite these limitations, the rapid evolution of new technologies continues at an unprecedented pace. Such progress, coupled with further efforts in basic and translational research, holds promise for elucidating the biological mechanisms behind the observations so far discussed, thereby improving predictions of neoadjuvant treatment responses in early-stage HER2- BC.

Funding

This review article did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of competing interest

CCo reports travel/accommodations (to scientific meeting) from Veracyte, past support by the IEO-Monzino Foundation (2023), and is supported by Fondazione Gianni Bonadonna (FGB) and Associazione Italiana per la Ricerca contro il Cancro (AIRC) (2024-2026). All the competing interests were outside the submitted work. BBK, BK, TR, FC, LP and SKK have no potential conflicts of interest to disclose. GB reports fees for advisory boards, travel grants, consultancy: Seagen, Eli Lilly, Novartis, Pfizer, Roche, AstraZeneca, MSD, Daiichi Sankyio, Eisai, Gilead, Exact Science, Stemline, Agendia. All the competing interests were outside the submitted work. CCr reports personal fees for consulting, advisory role and speakers' bureau from Lilly, Roche, Novartis, MSD, Seagen, Gilead and Pfizer. All the competing interests were outside the submitted work. GC reports the following honoraria for speaker's engagement: Roche, Seattle Genetics, Novartis, Lilly, Pfizer, Foundation Medicine, NanoString, Samsung, Celltrion, BMS, MSD; Honoraria for providing consultancy: Roche, Seattle Genetics, NanoString; Honoraria for participating in Advisory Board: Roche, Lilly, Pfizer, Foundation Medicine, Samsung, Celltrion, Mylan; Honoraria for writing engagement: Novartis, BMS; Honoraria for participation in Ellipsis Scientific Affairs Group; Institutional research funding for conducting phase I and II clinical trials: Pfizer, Roche, Novartis, Sanofi, Celgene, Servier, Orion, AstraZeneca, Seattle Genetics, AbbVie, Tesaro, BMS, Merck Serono, Merck Sharp Dome, Janssen-Cilag, Philogen, Bayer, Medivation, Medimmune. All the competing interests were outside the submitted work. ACG-C reports research funding (to the Institution) from AstraZeneca, Daiichi-Sankyo, Merck, Gilead Sciences, Zenith Epigenetics, Bristol-Myers Squibb, Novartis, Biovica International AB, and Foundation Medicine; and travel/accommodations (to scientific meeting) from Roche/Genentech. AGW institutional research support from Reveal Genomics, Genentech, Macrogenics, and Merck and personal fees from AstraZeneca outside the submitted work. EAM reports service on scientific advisory boards for Astra Zeneca, Exact Sciences, Roche/Genentech, and Merck; steering committee service for BMS, Lilly, and Roche/Genentech; and institutional research support from Roche/Genentech, Gilead, as well as research funding from Susan Komen for the Cure and participation as a member of the American Society of Clinical Oncology board of directors outside the submitted work. SMT reports a consulting or advisory role for Novartis, Pfizer, Merck, Eli Lilly, AstraZeneca, Genentech/Roche, Eisai, Sanofi, Bristol Myers Squibb, Seattle Genetics, CytomX Therapeutics, Daiichi-Sankyo, Gilead, OncXerna, Zymeworks, Zentalis, Blueprint Medicines, Reveal Genomics, ARC Therapeutics, Infinity Therapeutics, Sumitovant Biopharma, Umoja Biopharma, Artios Pharma, Menarini/Stemline, Aadi Biopharma, Bayer, Incyte Corp, Jazz Pharmaceuticals, Natera, Tango Therapeutics, Systimmune, eFFECTOR, and Hengrui USA; reports institutional research funding from Genentech/Roche, Merck, Exelixis, Pfizer, Lilly, Novartis, Bristol Myers Squibb, Eisai, AstraZeneca, Gilead, NanoString Technologies, Seattle Genetics, and OncoPep, and receives travel support from Eli Lilly, Sanofi, Gilead, and Pfizer. All the competing interests were outside the submitted work.

Acknowledgments

CCo contributed to the literature search, conception, design of the article and drafted the first version of the manuscript. BBK, BK, TR, FC, LP, SK contributed to the literature search and provided critical revisions of the manuscript. GB, CCr, GC, ACGC, AGW provided critical revisions of the manuscript. EAM and SMT provided critical revisions of the manuscript and supervision. Figure 1, Figure 2, and Figure 3 were created with biorender.com. All the authors provided final approval to the submitted work.

References

[1] Gennari A, André F, Barrios C, et al. ESMO Clinical Practice Guideline for the diagnosis, staging and treatment of patients with metastatic breast cancer. Ann Oncol 2021 Dec;32(12):1475–95.
[2] Cortazar P, Zhang L, Untch M, et al. Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis. Lancet 2014; 384:164–72.
[3] Schmid P, Cortes J, Dent R, et al. Event-free Survival with Pembrolizumab in Early Triple-Negative Breast Cancer. N Engl J Med 2022;386:556–67.
[4] Schmid P, Cortes J, Pusztai L, et al. Pembrolizumab for Early Triple-Negative Breast Cancer. N Engl J Med 2020;382:810–21.
[5] NCCN Clinical Practice Guidelines in Oncology. January 27, 2024. Accessed April 1, 2024. https://bit.ly/3HqLZ9t.
[6] Schmid P., Cortés J., Dent R., et al. LBO1-01: Neoadjuvant pembrolizumab or placebo plus chemotherapy followed by adjuvant pembrolizumab or placebo for early-stage triple-negative breast cancer: Updated event-free survival results from the phase 3 KEYNOTE-522 study. San Antonio Breast Cancer Symposium, 2023. San Antonio, TX.
[7] Schmid P, Cortes J, Dent R, et al. Overall Survival with Pembrolizumab in Early-Stage Triple-Negative Breast Cancer. N Engl J Med 2024.
[8] Mittendorf EA, Zhang H, Barrios CH, et al. Neoadjuvant atezolizumab in combination with sequential nab-paclitaxel and anthracycline-based chemotherapy versus placebo and chemotherapy in patients with early-stage triple-negative breast cancer (IMpassion031): a randomised, double-blind, phase 3 trial. Lancet 2020;396:1090–100.
[9] Gianni L, Huang CS, Egle D, et al. Pathologic complete response (pCR) to neoadjuvant treatment with or without atezolizumab in triple-negative, early high-risk and locally advanced breast cancer: NeoTRIP Michelangelo randomized study. Ann Oncol 2022;33:534–43.
[10] Gianni L., Huang C., Egle D., et al. LBA19 Event-free survival (EFS) analysis of neoadjuvant taxane/carboplatin with or without atezolizumab followed by an adjuvant anthracycline regimen in high-risk triple negative breast cancer (TNBC): NeoTRIP Michelangelo randomized study. Annals of Oncology, Volume 34, S1258 - S1259.

[11] Loibl S, Schneeweiss A, Huober J, et al. Neoadjuvant durvalumab improves survival in early triple-negative breast cancer independent of pathological complete response. Ann Oncol 2022;33:1149–58.
[12] Loibl S, Untch M, Burchardi N, et al. A randomised phase II study investigating durvalumab in addition to an anthracycline taxane-based neoadjuvant therapy in early triple-negative breast cancer: clinical results and biomarker analysis of GeparNuevo study. Ann Oncol 2019;30:1279–88.
[13] Sharma P, Stecklein SR, Yoder R, et al. Clinical and Biomarker Findings of Neoadjuvant Pembrolizumab and Carboplatin Plus Docetaxel in Triple-Negative Breast Cancer: NeoPACT Phase 2 Clinical Trial. JAMA. Oncol 2023.
[14] Kolberg H-C., Schumacher J., Erber R. PS16-01: Comparison of an Atezolizumab monotherapy window followed by Atezolizumab and chemotherapy vs. Atezolizumab and chemotherapy alone in triple negative breast cancer (TNBC) – final analysis of the neoadjuvant neoMono trial. San Antonio Breast Cancer Symposium, 2023. San Antonio, TX.
[15] Zdenkowski N., Loi S., Niman S., et al. LBO-03: Randomized Phase II Study of Neoadjuvant Nivolumab (N) 2 week lead-in followed by 12 weeks of concurrent N +carboplatin plus paclitaxel (CbP) vs concurrent N+CbP in Triple Negative Breast Cancer (TNBC): (BCT1902/IBCSG 61-20 Neo-N). San Antonio Breast Cancer Symposium, 2023. San Antonio, TX.
[16] Nanda R, Liu MC, Yau C, et al. Effect of Pembrolizumab Plus Neoadjuvant Chemotherapy on Pathologic Complete Response in Women With Early-Stage Breast Cancer: An Analysis of the Ongoing Phase 2 Adaptively Randomized I-SPY2 Trial. JAMA Oncol 2020;6:676–84.
[17] Pusztai L, Yau C, Wolf DM, et al. Durvalumab with olaparib and paclitaxel for high-risk HER2-negative stage II/III breast cancer: Results from the adaptively randomized I-SPY2 trial. Cancer Cell 2021;39:989–998.e985.
[18] Shatsky RA, Trivedi MS, Yau C, et al. Datopotamab-deruxtecan plus durvalumab in early-stage breast cancer: the sequential multiple assignment randomized I-SPY2.2 phase 2 trial. Nat Med 2024.
[19] Nederlof I, Isaeva OI, de Graaf M, et al. Neoadjuvant nivolumab or nivolumab plus ipilimumab in early-stage triple-negative breast cancer: a phase 2 adaptive trial. Nat Med 2024.
[20] Ignatidis M, Bailey A, McArthur HL, et al. GS01-03: Adding atezolizumab to adjuvant chemotherapy for stage II and III triple-negative breast cancer is unlikely to improve efficacy: interim analysis of the ALEXANDRA/IMpassion030 phase 3 trial. Cancer Res 2024;84(9_Supplement):GS01-03.
[21] Versluis JM, Long GV, Blank CU. Learning from clinical trials of neoadjuvant checkpoint blockade. Nat Med 2020;26:475–84.
[22] Grinshtein N, Bridle B, Wan Y, et al. Neoadjuvant vaccination provides superior protection against tumor relapse following surgery compared with adjuvant vaccination. Cancer Res 2009;69:3979–85.
[23] Blank C.U., Lucas M.W., Scolyer R.A., et al. Neoadjuvant nivolumab plus ipilimumab versus adjuvant nivolumab in macroscopic, resectable stage III melanoma: The phase 3 NADINA trial. JCO 2024;42:LBA2 - LBA2.
[24] Bianchini G, De Angelis C, Licata L, Gianni L. Treatment landscape of triple-negative breast cancer - expanded options, evolving needs. Nat Rev Clin Oncol 2021.
[25] Cortes J. Breast Cancer—Local/Regional/Adjuvant. Is More Better? Customizing Systemic Therapy for High-Risk, Nonmetastatic Breast Cancer. ASCO Annual Meeting 2024.
[26] Trapani D, Gandini S, Corti C, Crimini E, Bellerba F, Minchella I, et al. Benefit of adjuvant chemotherapy in patients with lobular breast cancer: A systematic review of the literature and metanalysis. Cancer Treat Rev 2021;97:102205.
[27] Johnston SRD, Harbeck N, Hegg R, et al. Abemaciclib Combined With Endocrine Therapy for the Adjuvant Treatment of HR+, HER2-, Node-Positive, High-Risk, Early Breast Cancer (monarchE). J Clin Oncol 2020;38:3987–98.
[28] Sparano JA, Gray RJ, Makower DF, et al. Adjuvant Chemotherapy Guided by a 21-Gene Expression Assay in Breast Cancer. N Engl J Med 2018;379:111–21.
[29] Kalinsky K, Barlow WE, Gralow JR, et al. 21-Gene Assay to Inform Chemotherapy Benefit in Node-Positive Breast Cancer. N Engl J Med 2021;385:2336–47.
[30] EBCTCG. Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. Lancet Oncol 2018;19:27–39.
[31] Anurag M, Zhu M, Huang C, et al. Immune Checkpoint Profiles in Luminal B Breast Cancer (Alliance). J Natl Cancer Inst 2020;112:737–46.
[32] Licata L, Barreca M, Galbardi B, et al. Breast cancers with high proliferation and low ER-related signalling have poor prognosis and unique molecular features with implications for therapy. Br J Cancer 2023;129:2025–33.
[33] Cardoso F, McArthur HL, Schmid P, et al. LBA21 KEYNOTE-756: Phase III study of neoadjuvant pembrolizumab (pembro) or placebo (pbo) + chemotherapy (chemo), followed by adjuvant pembro or pbo + endocrine therapy (ET) for early-stage high-risk ER+/HER2– breast cancer. Ann Oncol 2023;34(Supplement 2):S1260–1.
[34] Cardoso F, O'Shaughnessy J, McArthur HL, et al. Abstract GS01-02: Phase 3 study of neoadjuvant pembrolizumab or placebo plus chemotherapy, followed by adjuvant pembrolizumab or placebo plus endocrine therapy for early-stage high-risk ER+/HER2− breast cancer: KEYNOTE-756. Cancer Res 2024;84(9_Supplement):GS01-02.
[35] Loi S., Curigliano G., Salgado R., et al. LBA20 A randomized, double blind trial of nivolumab vs placebo with neoadjuvant chemotherapy followed by adjuvant endocrine therapy in patients with high risk, ER+ HER2− primary breast cancer. Annals of Oncology, Volume 34, S1259 - S1260.
[36] Rugo HS, Delord JP, Im SA, et al. Safety and Antitumor Activity of Pembrolizumab in Patients with Estrogen Receptor-Positive/Human Epidermal Growth Factor Receptor 2-Negative Advanced Breast Cancer. Clin Cancer Res 2018;24:2804–11.
[37] Rasmussen L, Arvin A. Chemotherapy-induced immunosuppression. Environ Health Perspect 1982;43:21–5.
[38] Ramakrishnan R, Assudani D, Nagaraj S, et al. Chemotherapy enhances tumor cell susceptibility to CTL-mediated killing during cancer immunotherapy in mice. J Clin Invest 2010;120:1111–24.
[39] Cortes J, Schöffski P, Littlefield BA. Multiple modes of action of eribulin mesylate: Emerging data and clinical implications. Cancer Treat Rev 2018;70:190–8.
[40] Goto W, Kashiwagi S, Asano Y, et al. Eribulin Promotes Antitumor Immune Responses in Patients with Locally Advanced or Metastatic Breast Cancer. Anticancer Res 2018;38:2929–38.
[41] Tolaney SM, Barroso-Sousa R, Keenan T, et al. Effect of Eribulin With or Without Pembrolizumab on Progression-Free Survival for Patients With Hormone Receptor-Positive, ERBB2-Negative Metastatic Breast Cancer: A Randomized Clinical Trial. JAMA Oncol 2020;6:1598–605.
[42] Shah AN, Flaum L, Helenowski I, et al. Phase II study of pembrolizumab and capecitabine for triple negative and hormone receptor-positive, HER2-negative endocrine-refractory metastatic breast cancer. J Immunother Cancer 2020, Feb;8 (1):e000173.
[43] Garrido-Castro A.C., Kim S.E., Desrosiers J., et al. SACI-IO HR+: A randomized phase II trial of sacituzumab govitecan with or without pembrolizumab in patients with metastatic hormone receptor-positive/HER2-negative breast cancer. JCO 2024 42:17_suppl, LBA1004-LBA1004.
[44] Szekely B, Bossuyt V, Li X, et al. Immunological differences between primary and metastatic breast cancer. Ann Oncol 2018;29:2232–9.
[45] Hofherr M., Hedgecorth J., Ademuyiwa F.O., et al. P3-06-06: Real-world analysis of adverse events of patients with triple negative breast cancer receiving therapy per KEYNOTE-522. San Antonio Breast Cancer Symposium, 2023. San Antonio, TX.
[46] Duma N, Lambertini M. It Is Time to Talk About Fertility and Immunotherapy. Oncologist 2020;25:277–8.
[47] Antonarelli G, Giugliano F, Corti C, et al. Research and Clinical Landscape of Bispecific Antibodies for the Treatment of Solid Malignancies. Pharmaceuticals (Basel) 2021;14.
[48] Ercoli G, Lopez G, Ciapponi C, et al. Building Up a High-throughput Screening Platform to Assess the Heterogeneity of HER2 Gene Amplification in Breast Cancers. J Vis Exp 2017.
[49] Chakraborty B, Byemerwa J, Krebs T, et al. Estrogen Receptor Signaling in the Immune System. Endocr Rev 2023;44:117–41.
[50] Holinka CF, Diczfalusy E, Coelingh, et al. Estetrol: a unique steroid in human pregnancy. J Steroid Biochem Mol Biol 2008;110:138–43.
[51] Kimbung S, Chang CY, Bendahl PO, et al. Impact of 27-hydroxylase (CYP27A1) and 27-hydroxycholesterol in breast cancer. Endocr Relat Cancer 2017;24: 339–49.
[52] Nelson AW, Groen AJ, Miller JL, et al. Comprehensive assessment of estrogen receptor beta antibodies in cancer cell line models and tissue reveals critical limitations in reagent specificity. Mol Cell Endocrinol 2017;440:138–50.
[53] Corti C, De Angelis C, Bianchini G, et al. Novel endocrine therapies: What is next in estrogen receptor positive, HER2 negative breast cancer? Cancer Treat Rev 2023;117:102569.
[54] Ladd JJ, Chao T, Johnson MM, et al. Autoantibody signatures involving glycolysis and splicesome proteins precede a diagnosis of breast cancer among postmenopausal women. Cancer Res 2013;73:1502–13.
[55] Pala L, De Pas T, Catania C, et al. Sex and cancer immunotherapy: Current understanding and challenges. Cancer Cell 2022;40:695–700.
[56] Haupt S, Caramia F, Klein SL, et al. Sex disparities matter in cancer development and therapy. Nat Rev Cancer 2021;21:393–407.
[57] Rudd KE, Johnson SC, Agesa KM, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the Global Burden of Disease Study. Lancet 2020;395:200–11.
[58] Martin GS, Mannino DM, Eaton S, et al. The epidemiology of sepsis in the United States from 1979 through 2000. N Engl J Med 2003;348:1546–54.
[59] Han R, Gu S, Zhang Y, et al. Estrogen promotes progression of hormone-dependent breast cancer through CCL2-CCR2 axis by upregulation of Twist via PI3K/AKT/NF-κB signaling. Sci Rep 2018;8:9575.
[60] Welte T, Zhang XH, Rosen JM. Repurposing Antiestrogens for Tumor Immunotherapy. Cancer Discov 2017;7:17–9.
[61] De Palma M, Lewis CE. Macrophage regulation of tumor responses to anticancer therapies. Cancer Cell 2013;23:277–86.
[62] Mor G, Yue W, Santen RJ, et al. Macrophages, estrogen and the microenvironment of breast cancer. J Steroid Biochem Mol Biol 1998;67:403–11.
[63] Seillet C, Laffont S, Trémollières F, et al. The TLR-mediated response of plasmacytoid dendritic cells is positively regulated by estradiol in vivo through cell-intrinsic estrogen receptor α signaling. Blood 2012;119:454–64.
[64] Paharkova-Vatchkova V, Maldonado R, Kovats S. Estrogen preferentially promotes the differentiation of CD11c+ CD11b(intermediate) dendritic cells from bone marrow precursors. J Immunol 2004;172:1426–36.
[65] Douin-Echinard V, Laffont S, Seillet C, et al. Estrogen receptor alpha, but not beta, is required for optimal dendritic cell differentiation and [corrected] CD40-induced cytokine production. J Immunol 2008;180:3661–9.
[66] Molloy EJ, O'Neill AJ, Grantham JJ, et al. Sex-specific alterations in neutrophil apoptosis: the role of estradiol and progesterone. Blood 2003;102:2653–9.
[67] Bain BJ, England JM. Normal haematological values: sex difference in neutrophil count. Br Med J 1975;1:306–9.

[68] Bain BJ, England JM. Variations in leucocyte count during menstrual cycle. Br Med J 1975;2:473–5.
[69] Mathur S, Mathur RS, Goust JM, et al. Cyclic variations in white cell subpopulations in the human menstrual cycle: correlations with progesterone and estradiol. Clin Immunol Immunopathol 1979;13:246–53.
[70] Dai R, Cowan C, Heid B, et al. Neutrophils and neutrophil serine proteases are increased in the spleens of estrogen-treated C57BL/6 mice and several strains of spontaneous lupus-prone mice. PLoS One 2017;12:e0172105.
[71] Zawrotniak M, Rapala-Kozik M. Neutrophil extracellular traps (NETs) - formation and implications. Acta Biochim Pol 2013;60:277–84.
[72] El-Benna J, Hurtado-Nedelec M, Marzaioli V, et al. Priming of the neutrophil respiratory burst: role in host defense and inflammation. Immunol Rev 2016;273:180–93.
[73] Vazquez Rodriguez G, Abrahamsson A, Jensen LD, et al. Estradiol Promotes Breast Cancer Cell Migration via Recruitment and Activation of Neutrophils. Cancer Immunol Res 2017;5:234–47.
[74] Abrahamsson A, Rodriguez GV, Dabrosin C. Fulvestrant-Mediated Attenuation of the Innate Immune Response Decreases ER. Cancer Res 2020;80:4487–99.
[75] Kropf P, Baud D, Marshall SE, et al. Arginase activity mediates reversible T cell hyporesponsiveness in human pregnancy. Eur J Immunol 2007;37:935–45.
[76] Ozerova M, Nefedova Y. Estrogen promotes multiple myeloma through enhancing the immunosuppressive activity of MDSC. Leuk Lymphoma 2019;60:1557–62.
[77] Toor SM, Syed Khaja AS, El Salhat H, et al. Myeloid cells in circulation and tumor microenvironment of breast cancer patients. Cancer Immunol Immunother 2017;66:753–64.
[78] Conforti F, Pala L, Bagnardi V, et al. Cancer immunotherapy efficacy and patients' sex: a systematic review and meta-analysis. Lancet Oncol 2018;19:737–46.
[79] Litchfield K, Reading JL, Puttick C, et al. Meta-analysis of tumor- and T cell-intrinsic mechanisms of sensitization to checkpoint inhibition. Cell 2021;184:596–614.e514.
[80] Unger JM, Vaidya R, Albain KS, et al. Sex Differences in Risk of Severe Adverse Events in Patients Receiving Immunotherapy, Targeted Therapy, or Chemotherapy in Cancer Clinical Trials. J Clin Oncol 2022;40:1474–86.
[81] Bayik D, Zhou Y, Park C, et al. Myeloid-Derived Suppressor Cell Subsets Drive Glioblastoma Growth in a Sex-Specific Manner. Cancer Discov 2020;10:1210–25.
[82] Milette S, Hashimoto M, Perrino S, et al. Sexual dimorphism and the role of estrogen in the immune microenvironment of liver metastases. Nat Commun 2019;10:5745.
[83] Castro A, Pyke RM, Zhang X, et al. Strength of immune selection in tumors varies with sex and age. Nat Commun 2020;11:4128.
[84] Conforti F, Pala L, Pagan E, et al. Sex-Based Dimorphism of Anticancer Immune Response and Molecular Mechanisms of Immune Evasion. Clin Cancer Res 2021.
[85] Venanzi FM, Bini M, Nuccio A, et al. Sex dimorphism and cancer immunotherapy: May pregnancy solve the puzzle? Cancer Treat Rev 2023 Dec;121:102648.
[86] Loo K, Tsai KK, Mahuron K, et al. Partially exhausted tumor-infiltrating lymphocytes predict response to combination immunotherapy. JCI. Insight 2017;2.
[87] Sharma P, Kimler BF, O'Dea A, et al. Randomized Phase II Trial of Anthracycline-free and Anthracycline-containing Neoadjuvant Carboplatin Chemotherapy Regimens in Stage I-III Triple-negative Breast Cancer (NeoSTOP). Clin Cancer Res 2021;27:975–82.
[88] Loi S, Salgado R, Adams S, et al. Tumor infiltrating lymphocyte stratification of prognostic staging of early-stage triple negative breast cancer. npj Breast Cancer 2022;8:3.
[89] Fanucci KA, Bai Y, Pelekanou V, et al. Image analysis-based tumor infiltrating lymphocytes measurement predicts breast cancer pathologic complete response in SWOG S0800 neoadjuvant chemotherapy trial. npj Breast Cancer 2023;9:38.
[90] Martín M, Yoder R, Salgado R, et al. Tumor-infiltrating lymphocytes refine outcomes in triple-negative breast cancer treated with anthracycline-free neoadjuvant chemotherapy. Clin Cancer Res 2024 May 15;30(10):2160–9.
[91] Dieci MV, Vernieri C, Massa D, et al. TILs as an independent prognostic factor for TNBC patients achieving pCR after NACT. San Antonio Breast Cancer. Symposium 2023.
[92] Leon-Ferre RA, Jonas SF, Salgado R, et al. Tumor-Infiltrating Lymphocytes in Triple-Negative Breast Cancer. JAMA 2024;331:1135–44.
[93] Wang XQ, Danenberg E, Huang CS, et al. Spatial predictors of immunotherapy response in triple-negative breast cancer. Nature 2023;621:868–76.
[94] Keren L, Bosse M, Marquez D, et al. A Structured Tumor-Immune Microenvironment in Triple Negative Breast Cancer Revealed by Multiplexed Ion Beam Imaging. Cell 2018;174:1373–1387.e1319.
[95] Tarantino P, Corti C, Schmid P, et al. Immunotherapy for early triple negative breast cancer: research agenda for the next decade. npj Breast Cancer 2022 Feb 18;8(1):23.
[96] Azizi E, Carr AJ, Plitas G, et al. Single-Cell Map of Diverse Immune Phenotypes in the Breast Tumor Microenvironment. Cell 2018;174:1293–1308.e1236.
[97] Virassamy B, Caramia F, Savas P, et al. Intratumoral CD8-positive T cells with a tissue-resident memory phenotype mediate local immunity and immune checkpoint responses in breast cancer. Cancer Cell 2023;41(3):585–601.e8.
[98] Savas P, Virassamy B, Ye C, et al. Single-cell profiling of breast cancer T cells reveals a tissue-resident memory subset associated with improved prognosis. Nat Med 2018;24:986–93.
[99] Karn T, Jiang T, Hatzis C, et al. Association Between Genomic Metrics and Immune Infiltration in Triple-Negative Breast Cancer. JAMA Oncol 2017;3:1707–11.
[100] Davoli T, Uno H, Wooten EC, et al. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science 2017. Jan 20;355(6322):eaaf8399.
[101] Wellenstein MD, Coffelt SB, Duits DEM, et al. Loss of p53 triggers WNT-dependent systemic inflammation to drive breast cancer metastasis. Nature 2019;572:538–42.
[102] Ghosh M, Saha S, Bettke J, et al. Mutant p53 suppresses innate immune signaling to promote tumorigenesis. Cancer Cell 2021;39:494–508.e495.
[103] Li Y, Sun Y, Kulke M, et al. Targeted immunotherapy for HER2-low breast cancer with 17p loss. Sci Transl Med 2021. Feb 10;13(580):eabc6894.
[104] Parkes EE, Walker SM, Taggart LE, et al. Activation of STING-Dependent Innate Immune Signaling By S-Phase-Specific DNA Damage in Breast Cancer. J Natl Cancer Inst 2016. Oct 5;109(1):djw199.
[105] Sharma P, Barlow WE, Godwin AK, et al. Validation of the DNA Damage Immune Response Signature in Patients With Triple-Negative Breast Cancer From the SWOG 9313c Trial. J Clin Oncol 2019;37:3484–92.
[106] Prestipino A, Zeiser R. Clinical implications of tumor-intrinsic mechanisms regulating PD-L1. Sci Transl Med 2019. Feb 6;11(478):eaav4810.
[107] Bachelot T, Filleron T, Bieche I, et al. Durvalumab compared to maintenance chemotherapy in metastatic breast cancer: the randomized phase II SAFIR02-BREAST IMMUNO trial. Nat Med 2021;27:250–5.
[108] Qin G, Wang X, Ye S, et al. NPM1 upregulates the transcription of PD-L1 and suppresses T cell activity in triple-negative breast cancer. Nat Commun 2020;11:1669.
[109] Bertucci F, Ng CKY, Patsouris A, et al. Genomic characterization of metastatic breast cancers. Nature 2019;569:560–4.
[110] Hutchinson KE, Yost SE, Chang CW, et al. Comprehensive Profiling of Poor-Risk Paired Primary and Recurrent Triple-Negative Breast Cancers Reveals Immune Phenotype Shifts. Clin Cancer Res 2020;26:657–68.
[111] Zhu L, Narloch JL, Onkar S, et al. Metastatic breast cancers have reduced immune cell recruitment but harbor increased macrophages relative to their matched primary tumors. J Immunother Cancer 2019;7:265.
[112] Stover DG, Parsons HA, Ha G, et al. Association of Cell-Free DNA Tumor Fraction and Somatic Copy Number Alterations With Survival in Metastatic Triple-Negative Breast Cancer. J Clin Oncol 2018;36:543–53.
[113] Rozenblit M, Huang R, Danziger N, et al. Comparison of PD-L1 protein expression between primary tumors and metastatic lesions in triple negative breast cancers. J Immunother Cancer 2020 Nov;8(2):e001558.
[114] Goldberg J, Pastorello RG, Vallius T, et al. The Immunology of Hormone Receptor Positive Breast Cancer. Immunol 2021 May;11(12):674192.
[115] Waks AG, Stover DG, Guerriero JL, et al. The Immune Microenvironment in Hormone Receptor-Positive Breast Cancer Before and After Preoperative Chemotherapy. Clin Cancer Res 2019;25:4644–55.
[116] Denkert C, von Minckwitz G, Darb-Esfahani S, et al. Tumour-infiltrating lymphocytes and prognosis in different subtypes of breast cancer: a pooled analysis of 3771 patients treated with neoadjuvant therapy. Lancet Oncol 2018;19:40–50.
[117] Watanabe T, Hida AI, Inoue N, et al. Abundant tumor infiltrating lymphocytes after primary systemic chemotherapy predicts poor prognosis in estrogen receptor-positive/HER2-negative breast cancers. Breast Cancer Res Treat 2018;168:135–45.
[118] Fujimoto Y, Watanabe T, Hida AI, et al. Prognostic significance of tumor-infiltrating lymphocytes may differ depending on Ki67 expression levels in estrogen receptor-positive/HER2-negative operated breast cancers. Breast Cancer 2019;26:738–47.
[119] Tesch ME, Guzman Arocho YD, Collins LC, et al. Association of tumor-infiltrating lymphocytes (TILs) with clinicopathologic characteristics and prognosis in young women with HR+/HER2- breast cancer (BC). JCO 2023;41:505. https://doi.org/10.1200/JCO.2023.41.16_suppl.505.
[120] Ríos-Hoyo A, Cobain E, Huppert LA, et al. Neoadjuvant Chemotherapy and Immunotherapy for Estrogen Receptor-Positive Human Epidermal Growth Factor 2-Negative Breast Cancer. J Clin Oncol 2024. JCO2302614.
[121] Leone P, Shin EC, Perosa F, et al. MHC class I antigen processing and presenting machinery: organization, function, and defects in tumor cells. J Natl Cancer Inst 2013;105:1172–87.
[122] Torigoe T, Asanuma H, Nakazawa E, et al. Establishment of a monoclonal anti-pan HLA class I antibody suitable for immunostaining of formalin-fixed tissue: unusually high frequency of down-regulation in breast cancer tissues. Pathol Int 2012;62:303–8.
[123] Garrido F, Ruiz-Cabello F, Cabrera T, et al. Implications for immunosurveillance of altered HLA class I phenotypes in human tumours. Immunol Today 1997;18:89–95.
[124] Kaneko K, Ishigami S, Kijima Y, et al. Clinical implication of HLA class I expression in breast cancer. BMC Cancer 2011;11:454.
[125] Park HS, Cho U, Im SY, et al. Loss of Human Leukocyte Antigen Class I Expression Is Associated with Poor Prognosis in Patients with Advanced Breast Cancer. J Pathol Transl Med 2019;53:75–85.
[126] Sinn BV, Weber KE, Schmitt WD, et al. Human leucocyte antigen class I in hormone receptor-positive, HER2-negative breast cancer: association with response and survival after neoadjuvant chemotherapy. Breast Cancer Res 2019;21:142.
[127] Lee HJ, Song IH, Park IA, et al. Differential expression of major histocompatibility complex class I in subtypes of breast cancer is associated with estrogen receptor and interferon signaling. Oncotarget 2016;7:30119–32.
[128] Kaklamanis L, Leek R, Koukourakis M, et al. Loss of transporter in antigen processing 1 transport protein and major histocompatibility complex class I

molecules in metastatic versus primary breast cancer. Cancer Res 1995;55:5191–4.
[129] Liu Y, Komohara Y, Domenick N, et al. Expression of antigen processing and presenting molecules in brain metastasis of breast cancer. Cancer Immunol Immunother 2012;61:789–801.
[130] Kajitani K, Tanaka Y, Arihiro K, et al. Mechanistic analysis of the antitumor efficacy of human natural killer cells against breast cancer cells. Breast Cancer Res Treat 2012;134:139–55.
[131] Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. Cell 2006;124:263–6.
[132] Medrek C, Pontén F, Jirström K, et al. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. BMC Cancer 2012;12:306.
[133] Tymoszuk P, Charoentong P, Hackl H, et al. High STAT1 mRNA levels but not its tyrosine phosphorylation are associated with macrophage infiltration and bad prognosis in breast cancer. BMC Cancer 2014;14:257.
[134] Bense RD, Sotiriou C, Piccart-Gebhart MJ, et al. Relevance of Tumor-Infiltrating Immune Cell Composition and Functionality for Disease Outcome in Breast Cancer. J Natl Cancer Inst 2017;109.
[135] Yang L, Huang F, Mei J, et al. Posttranscriptional Control of PD-L1 Expression by 17β-Estradiol via PI3K/Akt Signaling Pathway in ERα-Positive Cancer Cell Lines. Int J Gynecol Cancer 2017;27:196–205.
[136] Tuohy VK, Jaini R, Johnson JM, et al. Targeted Vaccination against Human α-Lactalbumin for Immunotherapy and Primary Immunoprevention of Triple Negative Breast Cancer. Cancers (Basel) 2016;8.
[137] Johnson M.J., Rhoades E.E., Levengood H., et al. PO2-17-12 : Phase I trial of alpha-lactalbumin vaccine in high-risk operable triple negative breast cancer (TNBC) and patients at high genetic risk for TNBC. San Antonio Breast Cancer Symposium, 2023. San Antonio, TX.
[138] Jaini R, Loya MG, Eng C. Immunotherapeutic target expression on breast tumors can be amplified by hormone receptor antagonism: a novel strategy for enhancing efficacy of targeted immunotherapy. Oncotarget 2017;8:32536–49.
[139] Márquez-Garbán DC, Deng G, Comin-Anduix B, et al. Antiestrogens in combination with immune checkpoint inhibitors in breast cancer immunotherapy. J Steroid Biochem Mol Biol 2019;193:105415.
[140] Tsao LC, Crosby EJ, Trotter TN, et al. CD47 blockade augmentation of trastuzumab antitumor efficacy dependent on antibody-dependent cellular phagocytosis. JCI Insight 2019;4.
[141] Cook KL, Soto-Pantoja DR, Clarke PA, et al. Endoplasmic Reticulum Stress Protein GRP78 Modulates Lipid Metabolism to Control Drug Sensitivity and Antitumor Immunity in Breast Cancer. Cancer Res 2016;76:5657–70.
[142] Cardoso F, van't Veer LJ, Bogaerts J, et al. 70-Gene Signature as an Aid to Treatment Decisions in Early-Stage Breast Cancer. N Engl J Med 2016;375:717–29.
[143] Corti C, Cobanaj M, Marian F, et al. Artificial intelligence for prediction of treatment outcomes in breast cancer: Systematic review of design, reporting standards, and bias. Cancer Treat Rev 2022;108:102410.
[144] Sammut SJ, Crispin-Ortuzar M, Chin SF, et al. Multi-omic machine learning predictor of breast cancer therapy response. Nature 2022;601:623–9.
[145] Stover DG, Coloff JL, Barry WT, et al. The Role of Proliferation in Determining Response to Neoadjuvant Chemotherapy in Breast Cancer: A Gene Expression-Based Meta-Analysis. Clin Cancer Res 2016;22:6039–50.
[146] Kabraji S, Solé X, Huang Y, et al. AKT1(low) quiescent cancer cells persist after neoadjuvant chemotherapy in triple negative breast cancer. Breast Cancer Res 2017;19:88.
[147] Alves CP, Dey-Guha I, Kabraji S, et al. AKT1-low Quiescent Cancer Cells Promote Solid Tumor Growth. Mol Cancer Ther 2018 Jan;17(1):254–63.
[148] Gaglia G, Kabraji S, Rammos D, et al. Temporal and spatial topography of cell proliferation in cancer. Nat Cell Biol 2022;24:316–26.
[149] Kabraji S, Sole X, Huang Y, et al. AKT1-low quiescent cancer cells in ductal carcinoma in situ of the breast. npj Breast Cancer 2019 Mar;21(5):10.
[150] Grinshpun A, Russo D, Ma W, et al. Pure estrogen receptor antagonists potentiate capecitabine activity in ESR1-mutant breast cancer. npj Breast Cancer 2024;10:42.
[151] Cobain EF, Pusztai L, Graham CL, et al. Elucidating the immune active state of HR+HER2- MammaPrint High 2 early breast cancer. JCO 2024;42:506.
[152] Wolf D.M., Yau C., Campbell M.J., et al. Biomarkers predicting response to 5 immunotherapy arms in the neoadjuvant I-SPY2 trial for early-stage breast cancer (BC): Evaluation of immune subtyping in the response predictive subtypes (RPS). JCO 2023 41:16_suppl, 102-102.
[153] Conte B, Brasó-Maristany F, Hernández AR, et al. A 14-gene B-cell immune signature in early-stage triple-negative breast cancer (TNBC): a pooled analysis of seven studies. EBioMedicine 2024;102:105043.
[154] Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750–67.
[155] Lehmann BD, Jovanović B, Chen X, et al. Refinement of Triple-Negative Breast Cancer Molecular Subtypes: Implications for Neoadjuvant Chemotherapy Selection. PLoS One 2016;11:e0157368.
[156] Ring BZ, Hout DR, Morris SW, et al. Generation of an algorithm based on minimal gene sets to clinically subtype triple negative breast cancer patients. BMC Cancer 2016;16:143.
[157] Iwase T, Blenman KRM, Li X, et al. A Novel Immunomodulatory 27-Gene Signature to Predict Response to Neoadjuvant Immunochemotherapy for Primary Triple-Negative Breast Cancer. Cancers (Basel) 2021;13.
[158] Dugo M, Huang C-S, Egle D, et al. PD10-06: Predictive value of RT-qPCR 27-gene IO score and comparison with RNA-Seq IO score in the NeoTRIPaPDL1 trial. Cancer Res 2022;82(4_Supplement):PD10-06.