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REVIEW ARTICLE

Luminal Breast Cancer: Risk of Recurrence and Tumor-Associated Immune Suppression

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Abstract

Hormone-receptor positive (HR+) breast cancer (BC) (including the luminal A and the luminal B subtypes) is the most common type of tumor in women diagnosed with early-stage BC (EBC). It represents a highly heterogeneous subgroup that is characterized by different risks of relapse. The aim of this review is to discuss the possible role played by the immune response in predicting this risk, along with the most common clinical and pathological factors and molecular tools that have been developed and are already in use. As opposed to what has previously been observed in the most aggressive human epidermal growth factor receptor 2 (HER2)-positive and triple-negative breast cancer (TNBC) subtypes, a high proportion of tumor-infiltrating lymphocytes (TILs)—reflecting a spontaneous and pre-existing immune response to the tumor—has been linked to a worse prognosis in HR+ EBC. This work provides some immune biological rationale explaining these findings and provides the basics to understand the principal clinical trials that are testing immunotherapy in HR+ (luminal) BC.

1 Introduction

Breast cancer (BC) is the second leading cause of cancer death in women (with about 143,000 deaths per year in Europe) [1], and with over 2 million new cases worldwide in 2018, it is the most common tumor in women [2]. BC patients with a known stage are usually diagnosed early

Key Points

In luminal/HER2-negative tumors, a high proportion of tumor-infiltrating lymphocytes was a negative prognostic factor.

Several trials are currently testing the efficacy of immune checkpoint blockade in hormone-receptor positive breast cancer, and it is hoped that the results will confirm the potential therapeutic role of immunomodulation in this subgroup of patients.

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in 79–87% of cases (stage I or II), with 13–21% of diagnoses made at a late stage (stage III or IV). Up to 7% of BC patients have metastases at diagnosis (stage IV) [3–5]. Despite early diagnoses, particularly after the introduction of the mammographic screening and surgery that allows a cure of most cases of early-stage BC (particularly luminal tumors), recurrence still occurs.

Therefore, adjuvant treatments (i.e., radiotherapy (RT), chemotherapy (CT), hormone therapy (HT), and anti-human epidermal growth factor receptor 2 (HER2)-targeted therapies), aiming to eradicate early systemic dissemination of microscopic disease, are commonly used to reduce the risk of relapse. Despite this progress, about 15% of patients will present a loco-regional relapse (i.e., tumor localized in breast and/or regional lymph nodes (LNs)) in the following 5 years [6]. Seventy-eight percent of women are predicted to survive for 10 years or more, as shown by age-standardized net survival for patients diagnosed with BC during 2010–2011 in England and Wales (Cancer Research UK Cancer Survival Group, London School of Hygiene and Tropical Medicine. Personal communication, 2014) and 10-year overall survival (OS) is about 86% and 78%, respectively [7].

A variety of clinical, pathological and molecular tools are used nowadays for treatment decisions in the adjuvant setting in luminal BC (e.g., whether CT administration is appropriate or not, based on the risk of relapse). In addition, novel biomarkers are under evaluation. Among them, a high proportion of tumor-infiltrating lymphocytes (TILs), as assessed on hematoxylin and eosin (H&E)-stained slides, in luminal primary BC was associated with a worse outcome, this is different from what was observed in the most infiltrated and aggressive BC subtypes (HER2-positive (HER2+) and triple negative BC (TNBC)), where high TILs predicted a better prognosis [8]. Thus, it could be speculated that, as opposed to HER2+ and TNBC, an efficient immune escape might represent one of the factors influencing recurrence in hormone receptor (HR)+ (= luminal) EBC.

Further, cancer immunotherapy through immune checkpoint blockade (ICB) has recently gained some success particularly in the treatment of TNBC patients in the metastatic and neoadjuvant settings [9, 10]. By incorporating immunotherapy into the standard TNBC treatment the revolution has been initiated, bringing new challenges, such as the assessment of responses to treatments with different timings and heterogeneous patterns of toxicity in a variety of organs [11–15], as well as the biggest issues of patient selection and the identification of ideal combinational drugs that might enhance the efficacy of ICB [9, 16–20]. In this light, luminal BC still remains an orphan with regard to immunotherapy options. It is becoming clearer that the heterogeneity of the various BC subtypes (luminal vs. HER2+ vs. TNBC) possibly explains the marked diversity of the spontaneous immune-related mechanisms that are generated, making it likely that their manipulation through ICB or other strategies (i.e., vaccines) will vary depending on the subtype [21, 22].

The aim of this review was to investigate the prognostic and predictive roles of the tumor immune environment, particularly with regard to the adaptive immunity in HR+ EBC patients.

2 Prognostic and Predictive Factors in Early-Stage Breast Cancer (BC)

2.1 Prognostic Factors

There are several strong prognostic factors for recurrence in EBC: tumor size (= T) [23], LN involvement (= N) [24], histological tumor grade [25], and the degree of tumor proliferation (= Ki67) [26]. There is also strong evidence for specific clinical and pathological factors, such as in the case of inflammatory BC, which is associated with a worse outcome [27], whereas tubular and mucinous carcinomas have a better prognosis [28]. HR status is both a prognostic and a predictive factor in EBC [29, 30]. While positivity of HR is associated with better prognosis, tumors with an overexpression of HER2/neu, which are found in 16–19% of cases, have a significantly worse prognosis (i.e., disease-free survival (DFS) and OS) [31].

Moreover, in 2000 Perou et al. proposed the "molecular profiling in BC," categorizing tumors into four molecular subtypes: (1) luminal A (= ER+ and/or PR+ and HER2− and low level of Ki67), (2) luminal B (ER+ and/or PR+ and/or HER2+ and high level of Ki67), (3) HER2-enriched (ER− and PR−/HER2+), and (4) basal-like (ER− and PR− and HER2−) [32]. Based on the assessment of these molecular subtypes through gene-expression profiling, we might more precisely assess prognosis and improve the prediction of benefit from CT in luminal subtypes. Indeed, different median durations of survival with distant metastases were shown in Luminal A (= 2.2 years), Luminal B (= 1.6 years), Luminal/HER2+ (= 1.3 years), HER2 enriched (= 0.7 years), and basal-like (= 0.5 years) [33]. However, recent data from trials on cyclin-dependent kinases (CDK)4/6 inhibitors administered in metastatic luminal BC reveal that OS could be superior, up to 3 years, in this group of patients [34, 35]. In addition, distinct patterns of metastatic spread within the different BC subtypes were observed. With the only exception being in the basal-like subtype, bone was the most common site of metastases. A higher rate of brain, lung, and LN metastases was specifically observed in basal-like tumors [33].

So far, a variety of gene-expression-profiling methods, such as Oncotype DX [36], MammaPrint [37], PAM50 [38], Breast Cancer Index [39], PREDICT score [40, 41], IHC4-score [42, 43], Clinical Treatment Score (CTS) [44], Magee

equation [45], and EndoPredict [46] have been developed to increase the accuracy on the prediction of the risk of recurrence, in order to guide treatment decisions (mainly adjuvant CT vs. no) in luminal subtypes. Unfortunately, none of these tests are currently used in clinical practice. A genomic signature provides stratification for early versus late recurrences in HR+ EBC. However, it has been internally but not externally validated [47]. Usually, in HR+ HER2− tumors (luminal A and B), systemic adjuvant therapy is based on HT, which is different between pre- and post-menopausal women. While the luminal A subtype is usually associated with a very good prognosis and the systemic adjuvant therapy is most likely HT alone, luminal B HER2− tumors can benefit from adjuvant CT. Indeed, the decision on what type of adjuvant treatment should be used in order to avoid over- or under-treatment in clinical practice remains very challenging. For instance, the phase III prospective randomized clinical trial "Microarray In Node-negative Disease may Avoid Chemotherapy" (MINDACT) aimed to dissect the issue of omission of adjuvant CT in patients with "discordant features," which means having high clinical (based on the traditional clinicopathological criteria, i.e., T, grade, presence or absence of HR, LN involvement) but low genetic risk of recurrence (evaluated with the MammaPrint signature). The absolute benefit from adding CT in this selected patient population was less than 2% in 5-y OS (98.8% vs. 97.0%), and less than 5% in 5-y DFS (93.3% vs. 88.8%), although the trial was not powered for such comparisons [48]. Furthermore, in the HR+ population, despite—or perhaps "due to"—having very good prognosis, late relapses (i.e., taking place > 5 years after diagnosis) still occur depending on risk factors mentioned above, as well as on the "impact of treatment" [49]. Despite all these efforts, adjuvant CT is not able to reduce late recurrences in HR+ EBC. As a consequence, several randomized trials were performed to evaluate the benefit from extended adjuvant HT and justify its use in certain conditions [50–53]. However, the real benefit remains small, if any, and it is further reduced by the occurrence of adverse events [54]. Therefore, we return to the question of (1) how to identify patients with a high risk of late recurrence, and (2) how to treat them.

Ethier et al. conducted a systematic review with meta-analysis regarding the prognostic role of neutrophil-to-lymphocyte ratio (NLR) in breast cancer [55]. Fifteen studies comprising a total of 8,563 patients were included [55]. Higher NLR was associated with worse OS (HR 2.56, 95% CI 1.96–3.35; $p < 0.001$) and DFS (HR 1.74, 95% CI 1.47–2.07; $p < 0.001$) [55]. This association was similar in studies including only early-stage disease and those comprising patients with both early-stage and metastatic disease. NLR had greater prognostic value for DFS in HR− and HER2− breast cancer, but no subgroup showed an influence on the association between NLR and OS.

The tumor cell dormancy phenomenon could explain the capability of disseminated tumor cells (DTCs) that give rise to metastases. These cells create non-proliferating dormant micro metastases for long periods of time, through immune escape or switch to angiogenesis [56]. Kim et al. performed a 49-gene signature specific for the analysis of tumor-cell dormancy (gene profiles including tumor cell quiescence and angiogenic regulation) in both BC cell lines and primary BC tumors, with the aim of finding the correlation between the dormancy gene profile and BC outcome. They defined the dormancy score by considering upregulated as positive genes and downregulated as negative genes in dormant cells. Among HR+ tumors, a higher dormancy score was significantly associated with a lower hazard of metastasis. Remarkably, a correlation between the dormancy score and the survival in HR+ tumors was observed. This was not the case for HR− tumors. Additionally, by comparing the dormancy scores of luminal A versus luminal B tumors, it was shown that the median score was significantly lower in the luminal B compared to the luminal A tumors [57].

2.2 Predictive Factors

So far, only three validated predictive factors of benefit from standard (neo)-adjuvant treatments exist in EBC: (1) estrogen receptor (ER), (2) progesterone receptor (PgR), and (3) HER2 (over)-expression. These factors are routinely used for the selection of patients for HT (with the presence of ER+ and/or PgR+ tumors) and anti-HER2 therapies (in HER2 overexpressing tumors), respectively. The TAILORx trial demonstrated the negative predictive role to chemotherapy in early HR+ breast cancer for tumor with a recurrence score lower than 25 [58]. In any case, its application is still limited in clinical practice in European Countries.

3 The Tumor Microenvironment in Luminal BC

The tumor microenvironment (TME) includes a variety of non-immune and immune cells producing many factors that can drive a chronic inflammatory, differently balanced situation: either a pro- and an anti-tumor or pro-angiogenic tumor environment [59] (Fig. 1). Among the non-immune cells, the stromal components of the TME consist of cancer-associated fibroblasts (CAFs), endothelial cells, and pericytes. Immune cells are particularly abundant in the stroma and less numerous in intra-tumoral areas [59]. They are composed of macrophages [tumor-associated macrophages (TAMs)], dendritic cells (DCs), myeloid-derived suppressor cells (MDSC), natural killer (NKs) cells, mast cells (MCs), granulocytes, plus the cells of the adaptive immunity, B and T lymphocytes. Naïve T cells represent the minority, while

memory T cells are the majority of cells, including cytotoxic $CD8^+$ T cells (CTL) and different subsets of $CD4^+$ T helper (Th) and immunosuppressive regulatory T cells (Tregs) [60]. It is noteworthy that B and T lymphocytes can be organized in tertiary lymphoid structures (TLS) whose role in BC has not been clearly defined yet [61, 62]. One can speculate that, potentially, all of these cells could have different impacts at different times and/or phases of tumor progression to control the (pro- or anti-) balance of the TME.

Immune cells can have pro-tumor or anti-tumor activity depending on how they communicate with tumor cells and with the other immune cells via the "inflammatory cross-talk network." It is evident that the BC-TME is different within the various subtypes, due to their marked heterogeneity. Furthermore, luminal BC was classified into three subgroups, beyond Luminal A and Luminal B subtypes, using the expression of 130 immune-related genes: high-TIL, low-TIL, and high-Interferon Stimulated Genes (ISG) [63]. Thus, we can observe different clinical patterns, prognostic characteristics, and biologic behaviors [63].

The role of TME components in modulating the response to anti-estrogen therapy in HR+ BC has not been fully clarified. However, pre-clinical data validated several cytokines that drive resistance to HT (i.e., Fibroblast Growth Factor 2 (FGF2) and Neuregulin 1 (NRG1)) [64].

3.1 Clinical Significance of Tumor-Infiltrating Lymphocytes in HR+ BC

A standardized methodology for evaluating TILs is a prerequisite for integrating this parameter in standard histopathological practice, in a research setting as well as in clinical trials [59]. This approach was established by Salgado et al. in 2015 and recommends reporting TILs as the stromal compartment (= % stromal TILs) [59]. The denominator used to determine the % stromal TILs is the area of stromal tissue (i.e., area occupied by mononuclear inflammatory cells over total intratumoral and stromal area), not the number of stromal cells (i.e., fraction of total stromal *nuclei* that represents mononuclear inflammatory cell *nuclei*) [59].

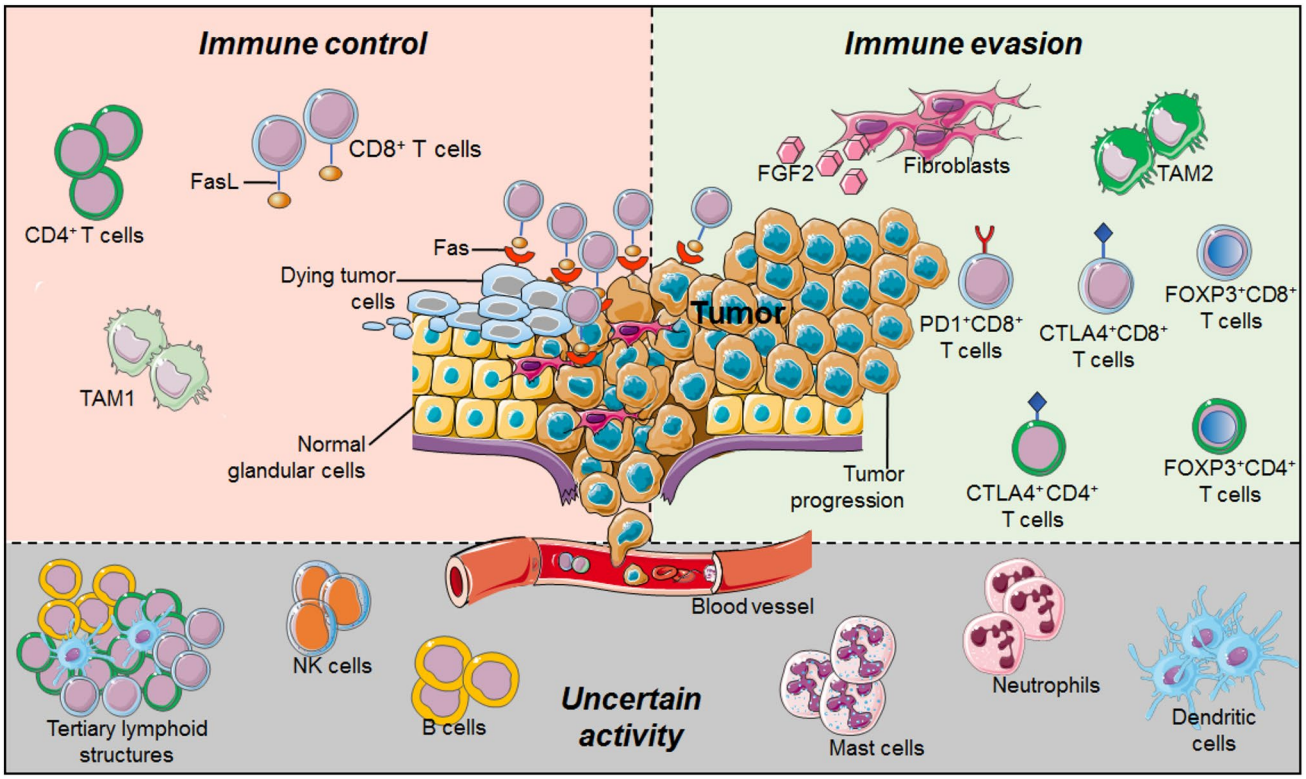

Fig. 1 The role of tumor microenvironment in hormone receptor-positive breast cancer. The tumor microenvironment of luminal breast cancer includes a variety of non-immune and immune cells producing many factors that can drive a chronic inflammatory, differently balanced situation: either a pro- and an anti-tumor or pro-angiogenic tumor microenvironment. *TAM-1* tumor-associated macrophages type 1, *TAM-2* tumor-associated macrophages type 2, *FGF2* fibroblast growth factor 2, *Fas-L* Fas ligand, *Fas* Fas receptor

Recently, Criscitiello et al. evaluated the extent of TILs in a retrospective mono-institutional case-cohort series of 987 patients with early ER+/HER2− BC [65]. In multivariable regression analysis, TILs correlate with higher Ki67 expression; at univariate Cox regression analysis, TIL level (≥ 5% vs. < 5%) was not associated with distant DFS (DDFS) ($p = 0.62$) [65]. Indeed, high TILs (≥ 5%) were associated with better DDFS only in patients treated with adjuvant chemotherapy ($p = 0.006$) [65].

Moreover, Denkert et al. showed that the presence of TILs assessed on H&E-stained slides is an independent predictor of response to neoadjuvant CT in all subtypes, particularly in TNBC and HER2-positive BC [66]. They further analyzed the association between the amount of TIL and the long-term outcomes (event-free survival and OS) in 2,560 patients with EBC undergoing neoadjuvant therapy. While increased TILs were associated with a survival benefit in HER2-positive and TNBC, in luminal/HER2-negative tumors high TIL was a negative prognostic factor, raising the question of the role played by the immune system in these tumors. Similar results were reached by Gao et al. and Waks et al., analyzing the association between pCR and survival, and TILs in HR+ BC [67, 68]. Further, another issue is how achieving a pathological complete response (pCR) correlates with long-term outcome in HR+ HER2-negative BC. Indeed, a recent meta-analysis by Spring et al. showed that patients with HR+ BC with pCR had only a trend towards a lower risk for recurrence versus patients without pCR [69]. A significant positive correlation was found between pCR and long-term outcomes for high-grade HR+ tumors only [69].

A meta-analysis of 25 studies comprising 22,964 patients showed that TILs improve OS in TNBC (HR 0.82; 95% CI 0.76–0.88 for DFS; HR 0.79; 95% CI 0.71–0.87 for OS) and HER2+ patients (HR 0.90; 95% CI 0.82–0.99 for DFS), but not in estrogen-receptor positive (ER+) patients (HR 1.01; 95% CI 0.94–1.07 for DFS; HR 1.09; 95% CI 0.98–1.21 for OS) [70].

Mahmoud et al. explored the prognostic value of tumor-infiltrating CTL in unselected BC patients with long-term follow-up [71]. They found a positive correlation between the total number of $CD8^+$ cells (= density) and higher grade, and a negative correlation with a patient's age at diagnosis, ER and PgR expression [71]. In HR− and basal phenotype tumors, remarkably the total $CD8^+$ counts were associated with better BC-specific survival, although in HR+ tumors the total number of infiltrating $CD8^+$ cells was not significantly associated with patient outcome [71].

3.2 FOXP Family and the Immune Response to BC

Preclinical studies showed that forkhead box P3 ($FOXP3^+$) regulatory T cells (Tregs) can inhibit the anti-tumor immune response [72]. In BC, tumor-infiltrating $FOXP3^+$ Tregs play a crucial role in immune escape [73, 74]. However, their prognostic value remains unclear. Mahmoud et al. analyzed the density of $FOXP3^+$ cells in a series of 1,445 cases of well-characterized primary invasive BC cases with long-term follow-up. The total number of $FOXP3^+$ cells showed a significant negative correlation with higher grade and HR. In addition, FOXP3 infiltration positively correlated with HER2 expression and the basal phenotype. The presence of intra-tumoral $FOXP3^+$ cells was associated with a worse prognosis on univariate analysis, but no significant prognostic role was found in multivariate analysis [75]. The meta-analysis performed in HR+ BC revealed lower OS in patients with high versus low tumor-infiltrating $FOXP3^+$ T cells but not in the HR− population [76]. Further, Liu et al. confirmed that the presence of high levels of $FOXP3^+$ TIL is associated with young age, high grade, HR negativity, concurrent $CD8^+$ TIL infiltration, HER2 positivity, and core basal subtypes. However, in HR+ BC lacking $CD8^+$ T-cell infiltrates, a high level of $FOXP3^+$ TIL was significantly associated with a poor survival [77]. This might signify that the balance between different subsets of immune cells can have a different impact on prognosis according to the various BC subtypes. Previously, Bates et al. showed that by quantification of $FOXP3^+$ Tregs we could identify patients at risk of late relapse within the HR+ subgroup [78].

An emerging member of the FOXP subfamily, FOXP1 (similar to the well-known FOXP3 whose role in regulatory T cells has been extensively studied [79]) is abnormally expressed in diverse human tumors including BC [80]. The *FOXP1* gene is located in a tumor suppressor locus at 3p14.1 and the loss of its expression in BC (as well as in endometrial, prostate, and renal cell carcinomas) has been associated with a worse outcome [81, 82].

In the immune system, information on FOXP1 is currently limited but rapidly growing, with studies showing that it functions as an essential transcriptional regulator of B cell lymphopoiesis [82] and plays a critical role in monocyte differentiation and macrophage function [83, 84]. In murine models, conditional deletion of the *FOXP1* gene in $CD4^+$ $CD8^+$ thymocytes revealed it is also essential for the generation of quiescent naïve T cells [85, 86].

This is also further elaborated by the work of Garaud et al., demonstrating the key role of FOXP1 in human peripheral blood $CD4^+$ T-cell quiescence and T-helper (Th) cell differentiation [87]. Further, Shi et al. found that FOXP1 upregulates the expression levels of cytotoxic T lymphocyte antigen -4 (CTLA-4) in conventional $CD4^+$ T cells abrogating their differentiation into follicular helper T cells *in vivo* [88]. With regard to FOXP1 expression in $CD8^+$ T cells, a study demonstrated that upregulation of FOXP1 in breast tumor-derived $CD8^+$ T cells drives T-cell unresponsiveness by blocking proliferation and major T-cell functions, including degranulation of cytotoxic granules and cytokine release.

This state, which is distinct from anergy and exhaustion, involves FOXP1 and Smad2/Smad3 interactions, both translocated to the nucleus in response to transforming growth factor-beta (TGFβ) signaling [89]. Moreover, a recent study showed that FOXP1 serves an essential function in Tregs to maintain their suppressive action by enforcing FOXP3-mediated regulation of gene expression [90].

FOXP1 is normally expressed by normal breast tissues and its expression is dysregulated in breast tumor epithelial cells [91]. Stromal cells also express FOXP1; these include fibroblasts, inflammatory cells, and endothelial and pericyte lining vessels. Over the past decades, several studies have demonstrated the significance of FOXP1 expression in BC and its link to prognosis since its discovery in 2001 [81]. FOXP1 may partly be regulated by ER expression, and it was shown previously that increased FOXP1 expression had a significant positive association with ER expression in primary human BC [91]. This correlation was further investigated to seek the transcriptional regulation of FOXP1 by ER in the ER+ MCF7 BC cell line where it was found that estrogen stimulation could significantly upregulate FOXP1 within a short time period [92]. Apart from ER regulation, a study demonstrated that it may be regulated via the PI3K/Akt/p70S6K signaling pathway [93]. Few studies showed that FOXP1 expression has been associated with better prognosis exploring in ER+ BC [91, 92, 94]. There is also information on breast cancer gene (*BRCA*) status and FOXP1, where negativity for FOXP1 was associated with a significantly worse OS in *BRCA2* cancers [94]. However, recent reports suggest *FOXP1* as an oncogene in ER− BC [93, 95].

3.3 CTLA-4: A Key Actor in Cancer Immune Evasion

CTLA-4 is predominantly expressed in FOXP3$^+$ Tregs or activated conventional T cells [96]. Expression of CTLA-4 was also described on CD8$^+$ Tregs [97] that are able to suppress the anti-tumor immune response by inhibiting the proliferation of effector T lymphocytes (participating in the regulatory mechanisms of interleukin (IL)-35) [98] and by inhibiting dependent allogenic responses [99].

Preclinical research on mice models injected with poorly immunogenic metastatic mouse mammary carcinoma 4T1 cells was performed to test the hypothesis of the efficacy of radiotherapy (RT) in primary tumor with CTLA-4 ICB. It was shown that RT alone blocked the progression of the primary tumor but in the absence of the anti-CTLA-4 monoclonal antibody (mAb) there was no benefit in survival. Indeed, CTLA-4 mAb alone did not have any effect on the primary tumor growth or survival. However, mice treated by combined therapy had a statistically significant survival benefit [100]. These observations suggest the role of possible systemic effects of RT when given in association with ICB [101].

The role of CTLA-4 expression was investigated by Mao et al. in breast tissues. The immunohistochemical (IHC) staining and reverse transcriptase polymerase chain reaction (RT-PCR) were performed in 60 BC patients and in 30 normal controls. The strong expression of CTLA-4 at both protein and mRNA levels was detected in tumor cells and a higher mRNA level of CTLA-4 was associated with worse LN involvement [102].

Further, in primary breast tumor tissues a significant hypomethylation of CpG islands in the promoter region of CTLA-4 compared with normal tissues was observed, signifying an upregulation of the *CTLA-4* gene [103].

At the protein level, expression of CTLA-4 by IHC was found in both immune cells (at the cytoplasmic level) and tumor cells in around 50% of BC analyzed in a retrospective study including 93 tumors [104]. Interestingly, another retrospective study analyzed the clinical impact of the expression of CTLA-4 by different cells of the TME in BC showing that the group of patients with high CTLA-4 expression on interstitial lymphocytes and with a low CTLA-4 expression on tumor cells had the best outcomes (DFS and OS) [105]. CTLA-4 expression in both tumor cells and TILs was associated with worse DFS and OS in luminal B HER2− BC [106]. A flow cytometry study investigating the intracellular protein expression of CTLA-4 by BC CD4$^+$ TILs revealed its almost low expression in the luminal versus HER2-positive and TNBC subtypes, as a consequence of their lower baseline immune infiltration [107].

3.4 Highlights of PD1/PD-L1 and HR+ BC

One of the well-recognized immune-checkpoints associated with immune evasion in BC is programmed cell death-1 (PD-1) and its ligand PD-L1 axis [108]. PD-1, which is mainly expressed on the surface of the T-cell membrane, when combined with PD-L1 induces T-cell apoptosis and promotes T-cell differentiation towards Tregs [109].

Innate absence of PD-L1 expression is associated with immune hyperactivity against self, such as that observed in autoimmune diseases such as systemic lupus erythematosus [110]. Consequently, it has been thought that the upregulation of PD-L1 in tumor cells (a rare phenomenon in BC) could be related to tumor immune evasion [61, 111]. PD-L1 mRNA was found to be expressed in at least 20% of BC cells [112] and the majority of tumors that upregulated PD-L1 are HR− (52% vs. 48%) [112, 113]. Many studies have relatively consistently reported that PD-L1 represents a good survival prognostic factor in TNBC [114–119], whereas the clinical role of PD-L1 is not clear in HR+ patients [61]. About 8−45% of HR+ BC express PD-L1 [60]. Interestingly, Wu et al. recently demonstrated that in HR+ patients, PD-L1 expression was associated with better pCR ($p = 0.022$), but with poor DFS ($p = 0.018$) [120]. Considering that PD-L1

expression is positively correlated with the extent of TILs, these findings are in line with the meta-analysis by Denkert et al. revealing that high TILs are associated with worse survival outcomes in luminal BC. Further, a large comprehensive meta-analysis revealed that whereas in HER2-positive and TNBC we can observe a statistically significant correlation between pCR and survival, in HR+ BC, there is no correlation [121]. Moreover, PD-L1 seems to provide additional favorable prognostic value to 21- and 70-gene scores in HR+ BC [122].

Based on these considerations, we may deduce that high TIL luminal BC are more likely to respond to neo-adjuvant chemotherapy compared to low TIL BC, but present more aggressive features that confer a worse prognosis. The explanation of this phenomenon might be found by examining the composition of the immune infiltrate.

3.5 The Role of Fas/Fas-Ligand (FasL) Pathway in BC Immunoevasion

The role of Tregs in the process of immune evasion is crucial. Tumor cells release the chemokines that attract Tregs into the tumor. Once these suppressive cells are recruited, they start to inhibit the function of Th cells stimulated against cancer cells, thus having a pro-tumoral effect. For example, they can induce Th cell apoptosis activating the Fas-Fas Ligand (FasL) pathway.

Fas (also known as APO-1 or CD95) belongs to the subgroup of the tumor necrosis factor receptor (TNF-R) family that contains an intra-cellular "death domain," and can trigger apoptosis. Its physiological ligand, FasL (CD95L), is a member of the corresponding TNF cytokine family [123].

The Fas death receptor is displayed on the surfaces of several types of lymphocytes, and by the activation of this extrinsic apoptotic pathway (=Fas-FasL) CTL are destroyed. Tumor cells can produce and release soluble forms of the apoptosis-inducing protein CD95L (FasL, APO-1L, CD178), thus eliminating TIL and suppressing anti-tumor immune responses, a phenomenon called "tumor counter-attack" [124]. The upregulation of FasL often occurs following CT, from which tumor cells have attained apoptosis resistance [125].

Interestingly, in a TiRP melanoma model, Zhu J et al. showed that TIL apoptosis was mediated by polymorphonuclear-myeloid-derived suppressor cells (PM-MDSCs) through FasL. Similarly, Tregs trigger apoptosis of $CD8^+$ cells by the high expression of FasL determining immune tolerance [126].

Several studies suggest that the downregulation of Fas in EBC is associated with a worse prognosis [127]. Mottolese et al. revealed that the DFS was significantly longer in patients with Fas-positive tumors compared to the Fas-negative ones [128]. These results were further confirmed by Reimer et al. and Botti et al., who found that the FasL:Fas ratio > 1 was related to a significantly shorter DFS [129, 130].

Furthermore, T and FasL:Fas ratio were of independent predictive significance in the multivariate model for DFS and OS in that subgroup. Among postmenopausal patients (n = 148), these factors retained independent prognostic significance in the multivariate model for DFS [129]. Based on these considerations, we can conclude that the expression of FasL is associated with a worse prognosis in HR+ EBC.

4 Clinical Trials with Immune Checkpoint Blockade (ICB) in HR+ BC

Several early phase trials have tested the safety and efficacy of immunotherapy alone or in combination with other agents in luminal BC patients (Tables 1, 2). In the neoadjuvant setting, pembrolizumab—in combination with chemotherapy—increased the pCR rates, varying from 15 to 30%, depending on the chemotherapy backbone [131]. In the advanced setting, ORR ranges from 12% for pembrolizumab alone [132] to 34% in association with eribulin [133] and 29% when combined with the CDK4/6i, abemaciclib [134]. As maintenance therapy, durvalumab (i.e., anti-PD-L1 antibody) improved OS compared to chemotherapy in HR+ BC (21.7 vs. 17.9) [135].

In order to improve the efficacy of immune activation in this subset of patients, several studies are currently testing the efficacy of ICB in this subtype. They are summarized in Table 3. Most of the trials are administering combination regimens in order to synergize the ICB effect. In this context, anti-PD-L1 is provided with anti-CTLA4 monoclonal antibodies (mAbs) (NCT03132467, NCT03608865) or with oncolytic virus (i.e., in the NCT03802604 trial), administered with the aim of increasing the immune infiltration at the tumor site.

Scientists are also trying to use the immunomodulatory and antigenic exposure effect of CT in order to enhance the activity of ICB. Previous findings demonstrated that the therapeutic efficacy of doxorubicin treatment is dependent on IL-1b, IL-17, and interferon gamma (IFNγ) production, and $CD8^+$ cell recruitment. The efficacy of eribulin may be attributed to its biological effects on the immune system, such as the reduction of PD-L1 and FOXP3 expression [136, 137], shifting the balance from a pro- to an anti-tumor immune response. Furthermore, the immune-modulating effects of taxanes appear to synergize with ICB. In particular, the reduction in Tregs and MDSCs paired with the recruitment of T cells and mature DCs to the tumor could render ICB-induced T cells more effective within the TME [138].

Table 1 Completed clinical trials with immune checkpoint inhibitors in hormone receptor-positive early breast cancer

ClinicalTrials.gov Identifier	Setting	Phase	Treatment arm(s)	ORR (%)	Survival (months)	Grade 3/4 AE prevalence (%)
NCT01042379	Neoadjuvant	2	Pembrolizumab four-arm /placebo + paclitaxel followed by doxorubicin + ciclofosfamide	NR	pCR 30% vs. 13%	25
NCT01042379	Neoadjuvant	2	Pembrolizumab 8 weekly paclitaxel x 12 wks + pembrolizumab q3 wks x 4 followed by pembrolizumab q3 wks x 4	NR	Non TNBC pCR 15	NR

AE adverse event, *pCR* pathological complete response, *NR* not reported, *TNBC* triple negative breast cancer

Based on these considerations, the following trials are NCT03393845, NCT03225547, NCT02990845,

Table 2 Completed clinical trials with immune checkpoint inhibitors in hormone receptor-positive advanced breast cancer

ClinicalTrials.gov Identifier	Setting	Phase	Treatment arm(s)	ORR (%)	Survival (mths)	Grade 3/4 AE prevalence (%)
NCT02054806	2L+	1b	Pembrolizumab	12	mDOR 12 mPFS 1.8 mOS 8.6	16
NCT03051659	2L+	2	Pembrolizumab + eribulin vs. Eribulim	25 vs. 34	mPFS 4.1 vs. 4.2	54.6 vs
NCT02299999	1L or 2 L	2	In patients with CR/PR/SD after 6–8 CT cycles and no targetable molecular alteration randomization to durvalumab or maintenance CT	39.7 vs. 42.6	ITT mPFS 2.7 vs. 4.6 mOS 21.7 vs. 17.9	13.2 vs. 15.9
NCT02779751	2L	1b	Pembrolizumab + abemaciclib	28.6	NR	NR

AE adverse event, *PFS* progression-free survival, *OS* overall survival

testing the efficacy of CT in association with ICB, in both the early and the advanced settings of the HR+ disease. They are: NCT03515798, NCT02957968, NCT03815890, NCT03356860, NCT03875573, NCT02999477, NCT02018458, NCT03725059, NCT03591276, NCT03841747, NCT03222856, NCT03409198, NCT02614833. Furthermore, the radiation-induced activation of the immune system has been increasingly recognized in recent years, suggesting that RT could also elicit immune-mediated anti-tumor responses [139, 140]. In fact, the role of T lymphocytes in the local tumor control induced by RT was demonstrated in a murine fibrosarcoma model more than 30 years ago [139, 140]. Other studies have found that this immune-mediated anti-tumor effect of RT could also trigger the regression of metastatic tumors that were distant from the irradiated field, which is the so-called abscopal effect [139, 141]. Consequently, RT has been associated with ICB in several clinical trials enrolling HR+ BC patients (NCT03366844 and NCT03051672).

Moreover, the HT aromatase inhibitors—in particular letrozole—seem to reduce the presence of intratumoral FOXP3$^+$ Tregs [142]; in this context, several trials are administering ICB in combination with aromatase inhibitors (NCT03874325, NCT02997995, NCT02204098, NCT03804944, NCT02971748, NCT03879174, NCT02648477, NCT03430479, NCT03430466).

Preclinical evidence suggests that CDK4/6 inhibitors (CDK4/6i) promote anti-tumor immunity by increasing antigen processing and presentation [143]. They may also modulate NK cell activity, augment T-cell effector function, and markedly suppress the proliferation of Tregs. A phase Ib study of the anti-PD-1 pembrolizumab plus abemaciclib in heavily pretreated patients with PD-L1-positive HR+ advanced BC showed an acceptable safety profile and a clinical activity (overall response rate: 14.3% at 16 weeks with a 75% disease control rate) [144]. Considering these results, further studies are now ongoing combining ICB and CDK4/6i (NCT02778685, NCT03147287, NCT03294694).

5 Conclusions

In summary, TME has a peculiar role in HR+ BC which seems to differ from other BC subtypes. In particular, in luminal/HER2-negative tumors, high TIL extent was a negative prognostic factor, and the total number of infiltrating CD8$^+$ cells was not significantly associated with patient outcome. Conversely, high tumor-infiltrating FOXP3$^+$ T cells has been associated with shorter OS, identifying patients with high risk of late-relapse within this subgroup. CTLA-4

Table 3 Currently ongoing clinical trials with immune checkpoint inhibitors in hormone receptor-positive early breast cancer

ClinicalTrials.gov Identifier	Setting	Phase	Primary endpoint(s)	Treatment arm(s)
NCT03515798	Neoadjuvant	2	pCR, DLT	Experimental arm: pembrolizumab+(F)EC followed by paclitaxel. Control arm: (F)EC followed by paclitaxel
NCT02957968	Neoadjuvant	2	TIL	Decitabine+pembrolizumab followed by dose-dense ACx4 followed by paclitaxel weekly x12
NCT03395899	Neoadjuvant	2	2-fold Increase in GzmB+ CD8+ T cell levels	Control arm: atezolizumab. Three experimental arms: (1) atezolizumab + cobimetinib, (2) atezolizumab + ipatasertib, (3) atezolizumab + cobimetinib + bevacizumab
NCT03815890	Neoadjuvant	2	Immune activation after pre-operative nivolumab	Cohort 1: nivolumab; cohort 2: nivolumab+doxorubicin
NCT03132467	Neoadjuvant	1	Feasibility, Safety	Durvalumab+tremelimumab
NCT03356860	Neoadjuvant	1/2	Toxicity, pCR	Experimental arm: durvalumab + chemotherapy (paclitaxel then EC). Control arm: chemotherapy (paclitaxel then EC)
NCT03874325	Neoadjuvant	2	Rate mPEPI score of 0	Durvalumab + aromatase Inhibitor
NCT02997995	Neoadjuvant	2	pCR	Tremelimumab + exemestane, followed by durvalumab + exemestane
NCT03875573	Neoadjuvant	2	Safety, residual cancer burden	Control arm: paclitaxel followed by dose-dense doxorubicin-cyclophosphamide (ddAC) and pre-operative RT. Experimental arm 1: durvalumab + control arm treatment. Experimental arm 2: oleclumab + control arm treatment
NCT02999477	Neoadjuvant	1	Change in PD-L1 expression by IHC from baseline biopsy to biopsy after 2-week treatment	Pembrolizumab + nab-paclitaxel
NCT03366844	Neoadjuvant	1	Safety, changes in TIL	Pembrolizumab+RT
NCT02204098	Neoadjuvant	1	Safety	Control arm Cohort 1: neoadjuvant endocrine therapy. Experimental arm Cohort 2: neoadjuvant endocrine + mammaglobin-A DNA vaccine. Control arm Cohort 3: neoadjuvant chemotherapy. Experimental arm Cohort 4: neoadj chemotherapy + mammaglobin-A DNA vaccine.
NCT02018458	Neoadjuvant	1/2	Safety	DC vaccine + chemotherapy AC
NCT03802604	Neoadjuvant	2	Gene signature CD8 Tc	Talimogene laherparepvec + atezolizumab
NCT03804944	Neoadjuvant/Adjuvant	2	Safety, clinical and pathological RR	Arm 1: RT; Arm 2: RT + pembrolizumab; Arm 3: RT+CDX301; Arm 4: RT + pembrolizumab + CDX301 (all arms: Letrozole until surgery, and thereafter decided by the treating physician)
NCT03725059	Neoadjuvant/ Adjuvant	3	pCR, EFS	Experimental arm: Pembrolizumab + chemotherapy (KX/KA[E]C). Control arm: Placebo + chemotherapy (PX/PA[E]C)
NCT02971748	Adjuvant	2	DFS	Pembrolizumab + hormonal therapy
NCT03879174	Advanced	2	PFS, ORR	Pembrolizumab + tamoxifen
NCT03393845	Advanced	2	ORR	Pembrolizumab + fulvestrant
NCT03225547	Advanced	2	ORR	Pembrolizumab + mifepristone
NCT03591276	Advanced	1	Safe dose doxil, ORR	Pembrolizumab + pegylated liposomal doxorubicin
NCT03841747	Advanced	2	PFS, OS	Experimental arm: pembrolizumab + paclitaxel. Control arm: paclitaxel
NCT02990845	Advanced	1/2	PFS at 8 months	Pembrolizumab + exemestane + leuprolide

Table 3 (continued)

ClinicalTrials.gov Identifier	Setting	Phase	Primary endpoint(s)	Treatment arm(s)
NCT03222856	Advanced	2	CBR	Pembrolizumab + eribulin
NCT02778685	Advanced	2	ORR	Pembrolizumab + letrozole + palbociclib
NCT02648477	Advanced	2	Safety, ORR in cohort 1, ORR in cohort 2	Pembrolizumab + AI
NCT03566485	Advanced	1/2	DLT, MTD, RP2D, ORR	Arm 1: atezolizumab + cobimetinib; Arm 2: atezolizumab + idasanutlin
NCT03280563	Advanced	1/2	ORR	Control arm: fulvestrant. Experimental arm: atezolizumab-containing doublet or triplet combination (enotinostat/ipatasertib/ipatasertib + fulvestrant/fulvestrant/bevacizumab + ET) (stage 1). Subsequent triplet combination (stage 2)
NCT03409198	Advanced	2	Toxicity, PFS	Control arm: pegylated liposomal doxorubicin + cyclophosphamide. Experimental arm: pegylated liposomal doxorubicin + cyclophosphamide +ipilimumab + nivolumab
NCT03430479	Advanced	1/2	DLT	Nivolumab + radiotherapy + hormonal therapy
NCT03608865	Advanced	2	ORR	Durvalumab + tremelimumab
NCT03430466	Advanced	2	ORR	Durvalumab + tremelimumab + fulvestrant
NCT03147287	Advanced	2	PFS	Control arm: fulvestrant. Experimental arm 1: Palbociclib + fulvestrant. Exlerimental arm 2: avelumab + Palbociclib + fulvestrant
NCT02614833	Advanced	1/2	RP2D, PFS	Control arm: paclitaxel + placebo. Experimental arm: paclitaxel+IMP321
NCT03051672	Advanced	2	ORR	Pembrolizumab+RT
NCT03294694	Advanced	1	MTD/RP2D	Cohort A: Ribociclib + PDR001; cohort B: Ribociclib + PDR001 + fulvestrant

pCR pathological complete response, *TIL* tumor-infiltrating lymphocytes, *IHC* immunohistochemical, *DFS* disease-free survival, *PFS* progression-free survival,

expression in both tumor cells and TILs was associated with worse DFS and OS in luminal B HER2-negative BC. Similarly, PD-L1 expression was associated with poor DFS. Several studies suggest that the downregulation of Fas in HR+ EBC is also associated with worse prognosis.

Based on the previous observations, several trials are currently testing the efficacy of ICB in HR+ BC and results are awaited to confirm the potential therapeutic role of the immunomodulation in this subgroup of patient. As the majority of the observations regarding the TME and its role in HR+ BC are retrospective, we strongly encourage prospective, translational trials aimed to dissect this topic.

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