



Interaction between redox regulation, immune activation, and response to treatment in HER2+ breast cancer

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ABSTRACT

In HER2+ breast cancer (BC), neoadjuvant therapy represents an ideal scenario for translational research, considering pathological complete response (pCR) as an endpoint. In these patients, achieving pCR after neoadjuvant therapy is associated with a better prognosis. However, biomarkers are needed to tailor optimal treatment for each patient. Evaluating tumour-infiltrating lymphocytes (TILs) has gained attention in predicting pCR. In the context of metastatic disease, TILs also appear to play a role in predicting outcomes. The interaction between the presence of TILs and reactive oxygen species (ROS) remains an area to be explored. ROS are critical for tumour cell homeostasis, and different levels can trigger differential biological responses in cancer cells and their microenvironment. Nevertheless, the influence of ROS on treatment efficacy and prognosis in patients with HER2+ BC remains to be elucidated. In this article, we reviewed the interplay between treatment response, immune system activation, and ROS production in HER2+ BC and suggested novel areas of intervention and research. We also present a bioinformatic analysis demonstrating that the altered expression of several redox-related genes could be associated with the prevalence of immune cell populations in the tumour microenvironment and with patient survival. New biomarkers are thus suggested and should be further explored to tailor the best treatment to each patient.

1. Introduction

In oncology, two patients with tumours classified under the same histological and immunohistochemical profile may still show substantial molecular or genomic differences, affecting treatment outcomes. This individuality leads to uncertainty about patient outcomes. It is therefore important to understand the mechanisms of resistance to each therapy. In the case of HER2+ early breast cancer (BC), neoadjuvant therapy (NAT) constitutes the ideal scenario for translational therapeutic and biomarker investigation, considering the pathological complete response (pCR) as an endpoint [1–3]. In those patients, achieving pCR after NAT is associated with a better prognosis and longer survival [4]. However, biomarkers are needed to tailor the best treatment for each patient.

The treatment of HER2+ stage IV BC has significantly advanced with

the use of HER2-blocking antibodies, such as trastuzumab and pertuzumab, trastuzumab emtansine (T-DM1), and more recently trastuzumab-deruxtecan (T-DXd) [5,6]. Nevertheless, primary and secondary resistance to these treatments remains a significant challenge, with an overall survival (OS) of approximately 50–60 months [7]. Consequently, there is an urgent need to develop novel therapeutic strategies to overcome treatment resistance and better stratify patients who will ultimately benefit from these treatments.

Evaluation of the presence of tumour-infiltrating lymphocytes (TILs) has gained visibility in breast carcinoma [8]. The interaction between the presence of TILs and reactive oxygen species (ROS) is still an area to be investigated. ROS are crucial for neoplastic cell homeostasis, and different levels can elicit differential biological responses [9,10]. Elevated levels can interfere with T cell survival, proliferation, and function. The underlying biochemical mechanisms are complex and still

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need to be fully elucidated, mainly regarding the impact that redox fluctuations have on treatment response and the prognosis of patients with HER2+ breast carcinoma.

In this article, we reviewed the interplay between treatment response, immune system activation, and redox mechanisms in HER2+ BC and proposed several ROS-related genes as candidates to be further studied in this context.

2. Methods

2.1. Narrative review

Using a research process mirroring a systematic search, we retrieved information from PubMed databases considering the period between 15 January and 30 June, 2024. Papers were extracted using combinations of the following search terms: HER2+ breast cancer, oxidative stress, and immune system. Only full papers written in English were included. Literature was screened for the most up-to-date and clinically relevant information, with a particular focus on diagnosis, therapeutic protocols, and clinical outcomes. Preference was given to high-impact studies, recent guidelines, and key findings from pivotal trials in the field. The selected information was then extracted and summarized to be used for this review.

2.2. Bioinformatic analysis

The methodology followed is summarized in Fig. 1. A set of 727 genes was selected from Gene Ontology (GO), based on three keywords: "oxidation", "redox" and "oxidase". The TCGA HER2+ Breast Invasive Carcinoma (TCGA-BRCA) dataset was used to analyse the expression level and association of these genes with the overall survival (OS) of patients. The tumour and normal tissue gene expression levels and the association of each gene expression with the OS were analysed using Gene Expression Profiling Interactive Analysis (GEPIA2; <http://gepia2.cancer-pku.cn/#index>) [11]. Median was used as a cut-off to define low and high gene-expressing groups for Kaplan-Meier survival analysis. Differences were considered statistically significant when the Log-rank test $p < 0.05$. A Cox proportional hazard model was selected and individual hazard ratios (HR) were calculated and were expressed as Log10 (HR).

Differential expression between tumoural HER2+ BC and normal tissues was analysed for the 87 genes with a significant impact on HR. A Welch's *t*-test was performed for the selected genes using the TCGA-UALCAN platform (<http://ualcan.path.uab.edu/index.html>) [12]. Genes with a ratio of tumoural versus normal tissue expression <0.8 or >1.2 and statistically significant differences ($p < 0.05$) calculated by a Welch's *t*-test between both groups were selected.

The TCGA data-based platform TIMER2.0 (<http://timer.cistrome.org/>) [13] was used to evaluate the association between the expression of the selected 53 genes and the abundance of immune infiltrates in HER2+ BC patients, by the TIMER algorithm (adjusted according to tumour purity). TIMER calculates the abundance of six immune cell types based on a score of arbitrary units using linear least squares regression [14]. Statistical significance was considered when $p < 0.05$ using the Spearman correlation coefficient.

The association between expression of the selected genes with overall patient survival was evaluated for the 12 most commonly diagnosed cancers by location in the United States in 2024 [15] <https://seer.cancer.gov/statfacts/html/common.html#comparison>). HR and statistical significance from the Log-rank test ($p < 0.05$) were derived from TCGA datasets, including prostate adenocarcinoma (PRAD), lung adenocarcinoma and squamous cell carcinoma (LUAD & LUSC), colon and rectum adenocarcinomas (COAD & READ), skin cutaneous melanoma (SKCM), bladder urothelial carcinoma (BLCA), diffuse large B-cell lymphoma (DLBC), uterine corpus endometrial carcinoma and uterine carcinosarcoma (UCEC & UCS), kidney chromophobe, renal clear cell and papillary cell carcinoma (KICH & KIRC & KIRP), acute myeloid leukemia (LAML), pancreatic adenocarcinoma (PAAD), and thyroid carcinoma (THCA).

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For a comprehensive functional analysis, gene enrichment was conducted based on biological processes and molecular functions using Gene Ontology (GO) terms. The analysis was performed with ShinyGO v0.80 (<http://bioinformatics.sdbstate.edu/go/>) [16]. The top ten most significant GO terms were ranked by false discovery rate (FDR) and further sorted by fold enrichment. Only pathways containing a minimum of ten genes and with an FDR <0.05 were considered for both upregulated and downregulated gene groups. Redundant pathways were excluded, and the results were visualized through Sankey and dot plots generated by Splot (<https://www.bioinformatics.com.cn/en>) [17]. The STRINGdb v12.0 (<https://string-db.org/>) was used to construct a protein-protein interaction network of the proteins encoded by the genes. Only Biological Processes (Gene Ontology) and Reactome Pathways with an FDR <0.005 were considered.

3. HER2+BC

HER2 is a proto-oncogene located on chromosome 17 that codes for a tyrosine-kinase receptor belonging to the epidermal growth factor receptor (EGFR) family, consisting of EGF/ErbB1 (HER1), ErbB2 (HER2), ErbB3 (HER3) and ErbB4 (HER4). Each of these surface receptors is encoded by a specific gene and comprises an extracellular ligand-binding domain, a transmembrane, and an intracellular tyrosine kinase domain. In contrast to other members of this family, HER2 is an orphan receptor that lacks its own ligand and instead relies on two mechanisms for activation: heterodimerization with another member of its receptor family or homodimerization with another HER2 receptor, primarily occurring at high expression levels [18,19]. Heterodimers tend to generate more robust signals than homodimers, and those involving HER2 exhibit particularly high ligand binding and signalling capabilities. This is because HER2 adopts an open conformation that makes it the preferred dimerization partner among its family members [20]. These dimerization events trigger autophosphorylation of the tyrosine residues within the receptor cytoplasmic domain, which act as docking sites to other tyrosine kinases, initiating a signalling cascade leading to a diversity of biological effects [21]. Among these dimers, the HER2-HER3 heterodimer emerges as the most potent stimulator of downstream pathways, including the PI3K/Akt pathway, a central regulator of cell growth and survival. Other pathways frequently activated upon HER2 stimulation include the mitogen-activated protein kinase (MAPK) and the protein kinase C (PKC), leading to cell proliferation, survival, differentiation, invasion, and enhanced angiogenesis. Furthermore, HER2 dimerization promotes the misplacement and rapid degradation of the cell-cycle inhibitor p27Kip1 protein, facilitating cell-cycle progression. Additionally, HER2 can become transactivated

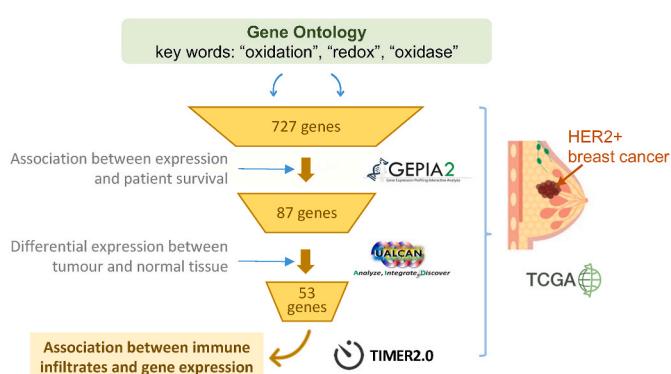


Fig. 1. Methodological approach used to explore the associations between the expression of redox-related genes with HER2+ BC patient survival and their effect on tumour infiltrates.

through interactions with extracellular matrix receptors, such as integrin $\alpha 6\beta 4$ [22], other tyrosine kinase receptors, like the insulin-like growth factor receptor 1 (IGF1R) or the hepatocyte growth factor receptor (HGFR or c-Met) [23], and of G-protein coupled receptors (GPCR), such as the protease-activated receptor 1 (PAR1) [24].

HER2 is overexpressed in about 40–60 % of the ductal carcinomas *in situ* and 15–30 % of the invasive breast carcinomas. In 90 % of the cases, overexpression is caused by gene amplification and rarely by occasional mutations or other chromosomal changes [25]. Until the beginning of 2000, HER2+ breast tumours were related to a worse prognosis, but the development of monoclonal antibodies against the ligand-binding domain of the receptor changed the paradigm of this disease. Trastuzumab was approved in 2000 by the European Medicines Agency and has shown clinical benefits in HER2+ metastatic breast cancer [26]. This drug was described to bind, through electrostatic and hydrophobic interactions, to three distinct regions of the subdomain IV of the HER2 receptor extracellular domain [27]. In an adjuvant setting, data from several randomized trials showed a significant improvement in disease-free survival (DFS) and OS in women with early HER2+ breast tumours [28]. Pertuzumab is a recombinant humanized monoclonal antibody that exclusively targets the extracellular subdomain II of HER2 and prevents its heterodimerization with other HER family members, such as EGFR, HER3, and HER4, impairing downstream signalling. Pertuzumab suppresses ligand-initiated intracellular signalling through MAPK and PI3K. Apoptosis and cell growth arrest can arise from the inhibition of these signalling pathways [29]. Such binding activates a signalling cascade that leads to Ca^{2+} release into the cytoplasm of immune effector cells, and the release of cytoplasmic granules, such as perforin and granzymes [29]. Additionally, trastuzumab and pertuzumab mediate antibody-dependent cellular cytotoxicity (ADCC), opsonizing the tumour cells that express the target HER2 receptors at the surface. While perforin opens pores in the cancer cell plasma membrane, it facilitates the diffusion of granzyme, which induces caspase activity and DNA damage, triggering apoptosis [30,31].

In early HER2+ BC, NAT constitutes the ideal scenario for translational therapeutic and biomarker investigation, considering the pCR as an endpoint. In those patients, achieving pCR after NAT is generally associated with a good prognosis and longer disease-free survival [3].

The current treatment for HER2+ BC with tumour size \geq cT2 and/or nodal disease is NAT based on dual HER2 blockade with trastuzumab and pertuzumab and chemotherapy [32]. This therapy is also considered in tumours between 1 and 2 cm with high-risk features, and as a way to enable converting a mastectomy into a conservative surgery. With this therapy, a DFS at 5 years of around 85 % can occur in patients who achieved pCR, and around 76 % for those patients who did not achieve pCR [33].

Numerous strategies have been investigated to either intensify or reduce systemic therapy in early-stage HER2+ BC, enhance the quality of life of the patient, optimize patient outcomes, and minimize unnecessary toxicity and costs. Examples include administering adjuvant therapy with T-DM1 to patients who do not achieve pCR following neoadjuvant therapy [34], or continuing pertuzumab in the adjuvant setting for patients who achieved pCR but initially presented nodal disease [35]. In this setting, novel biomarkers are needed to identify which patients are less likely to benefit from current therapies, select those who should enrol in clinical trials for different therapeutic approaches, and select patients for treatment de-escalation.

The treatment of HER2+ stage IV BC has significantly advanced with the use of HER2-blocking antibodies, such as trastuzumab and pertuzumab, in combination with chemotherapy, which has become the standard therapy for these patients [7]. However, despite the clinical benefits provided by these monoclonal antibodies, a substantial proportion of patients will progress. Antibody-drug-conjugates, such as T-DM1 [5] and trastuzumab-deruxtecan (T-DXd) [6], have shown efficacy in treating patients whose cancer has progressed after first-line therapy. Nevertheless, primary and secondary resistance to treatments

remains a significant challenge. In light of this, there is an urgent need to understand resistance mechanisms and to develop novel therapeutic strategies to overcome treatment resistance.

Despite the therapeutic benefits of anti-HER2 antibodies, some resistance mechanisms to these drugs have also been described. Activating mutations in the p110 α subunit of PI3K and/or inactivating mutations in PTEN play a crucial role in trastuzumab resistance through persistent activation of the PI3K/AKT pathway [36,37]. In addition, some BC cells express a truncated HER2 lacking the extracellular domain, preventing trastuzumab binding [38,39]. Furthermore, hyperactivation of other tyrosine kinase receptors, such as insulin-like growth factor-I receptor (IGF-IR), compensates for the trastuzumab inhibition of HER2 downstream signalling [40]. Single nucleotide polymorphisms in genes that code for Fc-gamma receptors (Fc γ Rs) have been suggested to reduce the response of BC patients to trastuzumab Fc-mediated ADCC [41].

4. The presence of tumour-infiltrating lymphocytes (TILs) in HER2+ BC

Tumour-infiltrating lymphocytes (TILs) are mononuclear immune cells, recruited from peripheral blood, that infiltrate the stroma of solid tumours to mount a local immune response to battle cancer cell proliferation, invasion, and metastasis. They essentially comprise CD4 $^+$ helper, CD8 $^+$ cytotoxic, T regulatory (Tregs), and B cells. However, the most recent single-cell sequencing and spatial transcriptomics data revealed that they may include a diversity of lymphoid subpopulations [42,43]. As one of the most abundant elements of the tumour microenvironment (TME), studying the behaviour and characteristics of TILs became crucial to understand tumour immune response dynamics and develop more effective immunotherapies against cancer.

The mutational burden of tumours, the hypoxic microenvironment, and the abnormal cancer cell metabolism are examples of factors that affect the recruitment of immune cells to the tumour site. Initially, innate immune cells are exposed to new antigens and mount an anti-tumour response that involves neutrophils, phagocytic macrophages, and dendritic cells with the ability to process and expose antigens at their surface, and to secrete peptides that recruit and activate TILs [44]. However, as the tumour progresses and cancer cells develop strategies to escape the immune surveillance, TILs accumulated at the TME may become dysfunctional and immunosuppressive. The most recent studies have taught us that not only the number but also the quality and spatial location of TILs within the TME may predict tumour outcome and patients' DFS and OS [45].

In general, the presence of TILs in tumours has been associated with a favourable response to treatment and a better prognosis in several tumour types, including BC. The evaluation of the presence of TILs has gained considerable visibility with several studies pointing them as an immunological biomarker predictive of response to NAT and with prognostic value, mainly in triple-negative and in HER2+ subtypes [46, 47]. A meta-analysis involving 4097 patients with early HER2+ BC found that higher TILs counts were associated with lower recurrence rates, with a 13% decrease in recurrence risk for every 10% increase in TILs [48]. Another study analysed the density of infiltrating CD8 $^+$ T cells in a cohort of 1334 patients with primary invasive BC, tracking their long-term progress. The findings revealed a significant correlation between high CD8 $^+$ lymphocyte counts and improved clinical outcomes, irrespective of other clinical factors, such as the HER2 status [49]. This was one of the first studies suggesting that CD8 $^+$ T cells exert anti-tumour activities and that enhancing their presence, through immunomodulatory strategies could be exploited as a therapeutic tool in BC treatment. Furthermore, increased levels of CD8 $^+$ T cells alongside the reduced presence of Forkhead box protein 3 (FOXP3) $^+$ T cells have been recognized as independent predictors of enhanced OS and recurrence-free survival (RFS) after treatment with neoadjuvant chemotherapy in both HER2+ and HER2- BC patients [50].

A high count of TILs on biopsy is related to an increased probability of obtaining a pCR after NAT [46,47]. Also, a recent meta-analysis of data from 4097 women across five trials revealed a significant association between high TILs and a reduced risk of relapse (adjusted HR per 10% increase in TILs: 0.87; 95% CI: 0.84–0.90; $p < 0.0001$). Notably, HER2+ tumours displayed lower TILs levels. The median TILs percentage was 13% (Interquartile Range: 5–30%), with fewer than 10% of patients exhibiting TILs exceeding 50% [45]. These findings highlight the importance of TILs in relapse risk and underscore the prevalence of lower TILs in HER2+ tumours. On the other hand, the importance of TILs presence in residual disease after surgery is not completely understood in HER2+ BC. Taking this information into account, the International Immuno-Oncology Biomarker Working Group published the protocol for the evaluation of TILs in residual disease to standardize procedures [51]. In Table 1, the findings from the available clinical studies addressing the levels of TILs in the residual disease of early HER2+ BC patients are summarized.

In the study developed by Kurozumi S. et al., high levels of TILs in patients who did not obtain a pCR were related to better prognosis [52]. An exploratory study evaluated the presence of TILs in the primary tumour and the residual disease of 175 patients with HER2+ BC submitted to neoadjuvant chemotherapy and trastuzumab: 78% of patients experienced a decrease in TILs after NAT, and this decrease was associated with a higher probability of achieving a pCR ($p < 0.001$). Interestingly, high levels of residual TILs were related to a decreased OS ($p = 0.009$) [53].

In a retrospective Italian cohort, the prognostic significance of TILs was assessed in 195 patients with HER2+ BC patients who did not achieve a complete response after neoadjuvant anti-HER2 chemotherapy. In this study, higher RD-TILs were significantly linked to worse OS when compared to lower RD-TILs (using a 15% cutoff). In patients who received trastuzumab therapy in the context of NAT, pCR was associated with a reduction in the TILs score after NAT [54]. This could be ascribed to a suppression of the immune response upon the removal of tumour cells. To the best of our knowledge, only two studies assessed TILs scores before and after HER2 blockage with trastuzumab and pertuzumab in HER2+ early BC. Griguolo et al [55] found a link between lower odds of pCR and greater stromal TILs at the time of surgery. A previous study from our group reported an association between pCR and the decrease of the TILs score after NAT [56].

It is not yet fully understood why high TILs in residual disease may be a factor of poor prognosis in HER2+ disease. NAT may induce an increase in immunosuppressive cells and a decrease in cytotoxic T cells. Among TILs subsets, cytotoxic CD8⁺ T cell infiltration is considered an indicator of effective antitumour immunity, and high CD8⁺ TILs are associated with favourable prognosis. Ladoire et al. [50] investigated post-treatment TILs in 111 HER2+ BC patients and showed that low levels of CD8⁺ infiltration after NAT were associated with poor RFS (HR = 3.85, $p < 0.0001$). CD4⁺ T cells also play an important role in regulating many aspects of adaptive immunity. Different subsets of CD4⁺ T cells can exhibit both pro-tumour and anti-tumour activities [57,58]. FOXP3⁺ regulatory T cells, also known as CD4⁺ CD25⁺ Tregs, have suppressive effects on anti-tumour immunity and have been associated with poor clinical outcomes [59,60]. Low levels of FOXP3⁺ cell infiltration after NAT were previously associated with better RFS (HR = 0.52, $p = 0.036$) [50]. It should be emphasised that most studies addressing TILs in residual HER2+ disease did not enrol patients treated with pertuzumab. Thus, we cannot predict the role of this agent in modulating immune activity. More studies are necessary to understand the impact of TILs in residual HER2+ disease in patients submitted to dual HER2 blockade. As previously mentioned, trastuzumab can lead to HER2+ BC cell death, by interfering with HER2 signalling but also via immune mechanisms such as ADCC and complement-dependent cytotoxicity, which increase the presence of intratumoural lymphocytes, namely CD8⁺ T cells and natural killer (NK) cells. When trastuzumab binds to HER2, the receptor is internalized and subsequently degraded.

Table 1

Summary of previous studies about TILs in early HER2+ BC in the neoadjuvant setting.

Reference	Study design (number of patients)	Neoadjuvant treatment	Changes between pre- and post-treatment TILs	Impact of residual TILs on prognosis
Kurozumi S et al. [52]	Retrospective analysis (n = 128)	Trastuzumab and chemotherapy	Stable TILs levels in 71.1% Decreasing TILs levels in 8.9%	High TILs in the residual disease in patients with non-pCR were related to a better prognosis ($p = 0.033$)
Hamy A-S et al. [53]	Retrospective analysis (n = 175)	Trastuzumab and chemotherapy	TILs levels decreased during treatment in 78% of the patients	A decrease in TILs level during NAT was associated with pCR. In patients with residual disease, TILs level >25% were associated with an adverse outcome (HR = 7.98, $p = 0.009$)
Miglietta F et al. [54]	Retrospective analysis (n = 195)	Trastuzumab and chemotherapy	Not reported	Higher RD-TILs were significantly linked to worse OS when compared to lower RD-TILs in patients with no-pCR (using a 15% cut-off)
Griguolo G et al. [55]	Retrospective analysis of a clinical trial (n = 151)	Trastuzumab and lapatinib	The most frequently observed pattern (n = 32, 26%) was an increase in TILs from baseline to day 15 followed by a decrease from day 15 to surgery	Not addressed
Luz P et al. [56]	Retrospective analysis (n = 30)	Trastuzumab, pertuzumab and chemotherapy	Trend to lower TILs scores in the residual disease compared with the biopsy. Among patients with decreased TILs, 11/17 (65%) had pCR after NAT	Not addressed

HR: hazard ratio; NAT: neoadjuvant therapy; OS: overall survival; pCR: pathological complete response; RD: residual disease; TILs: tumour-infiltrating lymphocytes.

Certain peptides resulting from this degradation are presented by proteins of the major histocompatibility complex I (MHCI) leading to the activation of cytotoxic T lymphocytes (antibody-dependent cellular phagocytosis) [61,62]. Dual-targeting of HER2 by concomitant use of trastuzumab and pertuzumab can modulate immune activation by a synergistic effect [59]. Particularly, pertuzumab significantly increased trastuzumab-induced ADCC in a model of uterine adenocarcinoma with low HER2 expression [63]. The combination of the two agents also enhanced the recruitment of NK cells for ADCC and delayed the outgrowth of xenografts from intrinsically trastuzumab-resistant cells [64]. These mechanisms are also enhanced by chemotherapy combinations. Cytotoxic agents (anthracyclines, taxanes, and cyclophosphamide) combined with anti-HER2 agents induce the release of tissue damage-associated molecular pattern molecules (DAMPs), which activate the immune system [65]. Some studies show that cytotoxic agents exert additional anti-tumour effects through induction of an immune response against the tumour by several mechanisms: formation of immunogenic epitopes, cytokine secretion, enhanced phagocytosis by macrophages, increased antigen-presentation and activation of dendritic cells, and augmented cytotoxic T cell function. In BC, chemotherapy with anthracyclines and taxanes can induce an anti-tumour immune response promoting the accumulation of lymphocytes in the TME [66]. Therefore, the presence of lymphocyte infiltrates in residual disease after therapy is a prognostic biomarker, but also reflects the immune reaction of the TME to chemotherapy.

In Table 2, a summary of previous studies examining the role of TILs in HER2+ metastatic BC is provided. Luen SJ et al. [67] conducted a retrospective analysis of the CLEOPATRA trial ($n = 678$), finding no significant association between TILs and progression-free survival (PFS), though a 10% increase in TILs was linked to improved OS. Pertuzumab addition showed no significant effect on PFS or OS. Liu S et al. [68] performed a retrospective analysis of the MA trial ($n = 427$), revealing that low CD8⁺ cytotoxic TILs were associated with worse PFS in lapatinib-treated patients, but found no association between TILs and PFS or OS.

A better understanding of the molecular mechanisms underlying the modulation of the immune response by anti-HER2 therapy is essential for the development of new therapeutic strategies and the identification of prognostic markers.

Table 2
Summary of previous studies about the role of TILs in HER2+ BC in the metastatic setting.

Reference	Study design (number of patients)	Treatment	Impact of pre-treatment TILs
Luen SJ et al. [67]	Retrospective analysis of a clinical trial - CLEOPATRA ($n = 678$)	Trastuzumab, pertuzumab and docetaxel ($n = 336$) vs Trastuzumab, placebo, and docetaxel ($n = 342$)	No significant association between TILs and PFS. A 10% increase in TILs was linked to improved OS. No association between TILs and PFS or OS upon pertuzumab addition
Liu, S et al [68]	Retrospective analysis of a clinical trial - MA ($n = 427$)	Trastuzumab and taxane vs Lapatinib and taxane	Low levels of CD8 ⁺ cytotoxic TILs were linked to poorer PFS in patients treated with lapatinib No association between TILs and PFS or OS

OS: overall survival; PFS: progression-free survival; TILs: tumour-infiltrating lymphocytes.

5. Oxidative stress and HER2+ BC

The concept of oxidative stress has been discussed and updated in the last decades. It comprises not only an imbalance between oxidants and antioxidants, in favour of the oxidants but also the disruption of redox signalling and regulation [69]. The role of different reactive species, mainly ROS, has been extensively studied, including in the context of cancer [69–71].

Cancer cells often experience higher levels of oxidative stress when compared to normal cells due to their increased metabolic activity and altered redox balance. There is evidence that supports a role for oxidative stress in the pathogenesis of cancer, since the homeostasis of neoplastic cells depends on the control of intracellular levels of ROS, and varying levels can result in different biological responses [70,72]. Low or moderate levels of ROS favour the activity of signalling pathways in neoplastic cells associated with cell proliferation and differentiation (Fig. 2). At low levels, ROS can act as secondary messengers by activating various proteins with kinase activity (e.g. PI3K/AKT, p38 MAPK, and ATM) and transcription factors (e.g. Nrf-2, NF- κ B, and HIF-1), which may favour the survival of neoplastic cells by stimulating cell proliferation, inflammation, invasion, and angiogenesis [73]. To adapt to high levels of ROS, cancer cells undergo several modifications, including in NADPH generation, sulphur-based metabolism, and activity of antioxidant transcription factors [70]. In the initiation stage, genetic alterations allow cell survival in the presence of high levels of ROS, for example, through the activation of antioxidant transcription factors. During tumour progression and metastasis, cancer cells further adjust to oxidative stress by increasing NADPH levels through multiple mechanisms [70]. However, the presence of levels of ROS above a certain threshold is incompatible with cell viability, even for a highly tumorigenic cell, leading to cell death [74].

Regarding BC, ROS have also been implicated in tumour development and progression. BC normally develops in a very pro-oxidative environment because of the fat tissue surrounding the breast gland. As a result, excess ROS immediately affects the lipidic neighbourhood and produces several active metabolites that can control different cellular activities. Malondialdehyde (MDA), 8-F2-isoprostanes, and 4-hydroxy-nonenal (4-HNE) are well-known examples of low-molecular-weight aldehydes that result from lipid peroxidation processes and that have been identified as potential indicators of the oxidative status in BC patients [75]. BC cells can escape cell death caused by high levels of ROS by increasing the levels of antioxidant defences, such as glutathione (GSH) [73,76].

Despite the notable evidence of oxidative stress and high levels of ROS in cancer cells, the research on the role of oxidative stress in HER2+ BC cells is limited. HER2 overexpression may enhance oxidative stress pathways to promote BC growth. The development of HER2+ BC may be influenced by some redox-sensitive signalling pathways [77]. HER2+ BC patients generally show increased superoxide dismutase (SOD) activity, which leads to the production of hydrogen peroxide and may promote tumour development and invasion [78]. HER2+ BC patients also show lower systemic GSH levels compared to HER2-patients, suggesting the involvement of oxidative stress in HER2+ BC [75]. Accordingly, a preliminary study by Santos et al. suggested that HER2+ BC patients presented lower serum antioxidant capacity when compared to HER2- BC or benign breast disease [79]. A recent study showed a significant increase in oxidative stress markers, such as ROS levels, MDA, and protein carbonylation in different HER2+ BC cell models. Moreover, it was also suggested that the oxidative stress observed in HER2+ cells could be a consequence of the oncogenic activity of HER2 receptor and of PI3K/Akt activation, leading to the induction of glycolysis [80].

HER2+ cells, similarly to other cancer cells, have higher levels of ROS making them more susceptible to further damage by increased ROS levels induced by pro-oxidant anticancer drugs. The higher reactivity and vulnerability of cancer cells offer an opportunity for many anti-

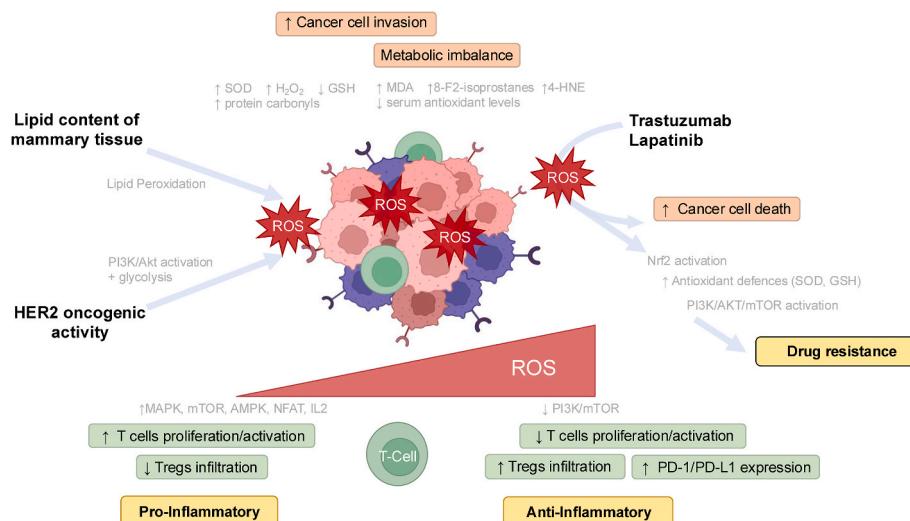


Fig. 2. Schematic representation of tumour redox status and its impact on the HER2+ breast cancer phenotype, drug sensitivity, and T-cell immune response.

cancer drugs and treatments. Therefore, in addition to their main mode of action, classic chemotherapeutic drugs with cytostatic activity often increase intracellular ROS [81]. Regarding the influence of anti-HER treatment on the production of ROS, some studies show that the binding of trastuzumab to the HER2 receptor leads to an increase in ROS in neoplastic cells and consequently to cell death. Moreover, anti-HER2 therapy leads to the activation of ROS-dependent cell death pathways that are implicated in cardiotoxic side effects [82]. Lapatinib is a tyrosine kinase inhibitor (TKI) approved for the treatment of HER2+ BC due to the important role of the receptor tyrosine kinase (RTK) in controlling cellular pro-survival and pro-proliferative signalling. Increased ROS levels have been reported after the treatment with Lapatinib. Additionally, low ROS levels and increased antioxidant defences, namely SOD and GSH, have been associated with the development of drug resistance [83]. The overexpression of HER2 results in an amplification of NRF2-dependent transcriptional activation, thereby elevating the expression of several proteins involved in detoxification and drug resistance, including Glutathione S-Transferase Alpha 2 (GSTA2), Glutathione S-Transferase Pi 1 (GSTP1), Cytochrome P450 3A4 (CYP3A4), hemoxygenase (HO-1), Multidrug resistance-associated protein 1 (MRP1), and Multidrug Resistance-Associated Protein 5 (MRP5) [84]. Moreover, the increase in antioxidant enzymes was identified as one of the cellular resistance mechanisms to anti-HER2 therapy. For example, Thioredoxin 1 (Trx-1) binds to and inhibits PTEN, favouring activation of the PI3K/AKT/mTOR pathway and consequently increasing cell proliferation of HER2+ BC cells treated with trastuzumab. Additionally, the treatment with the Trx-1 inhibitor 1-methylpropyl 2-imidazolyl disulfide (PX-12) re-established the sensitivity of BC cells to trastuzumab [85].

6. Redox regulation of immune infiltrates in HER2+ BC

In addition to cancer cells, oxidative stress has pronounced effects on the surrounding TME, affecting the survival and function of endothelial cells, fibroblasts, and immune cells [86]. One of the prime effects of ROS is to act as chemoattractants, enhancing the recruitment of distinct immune cell populations towards the tumour site. However, depending on its concentration, ROS may act as promoters or inhibitors of an effective inflammatory response (Fig. 2) [87]. The balance between the generation and consumption of ROS is a crucial element that influences T cell apoptosis, activation, differentiation, proliferation, and function, although the exact mechanisms are not well established [88].

At low to moderate concentrations, ROS are necessary for T cell activation, growth, and effector activity. In particular, ROS are

described to activate the MAPK pathway and its downstream targets, the mammalian target of rapamycin (mTOR) and the AMP-activated protein kinase (AMPK), promoting the survival and activation of T cells. ROS may also increase Ca²⁺ intracellular levels, promoting the activation of the transcription factor NFAT (nuclear factor of activated T cells) which induces interleukin 2 (IL-2) production, enhancing the proliferation of activated T cells [89]. Mitochondrial ROS are also able to control the activation of these cells by regulating the expression of IL-2 and IL-4 [90].

ROS differently modulate the activity of distinct T cells subpopulations. *In vitro* suppression of ROS by antioxidants or NOX inhibitors leads to a decrease in Treg cells favouring the activity of CD8⁺ T cells [88,90]. Reduced ROS production and reduced phosphorylation of JNK and NF-κB affected IFN-γ and CD39 expression in CD8⁺ T cells. In contrast, high levels of ROS inhibit the PI3K/mTOR signalling pathway, which is critical for T cell proliferation and activation. High levels of ROS are also able to affect the immunological synapse by inhibiting the recognition of the T cell receptor to proteins of the main histocompatibility complex, but also by promoting the expression of the immune checkpoints programmed death receptor 1 (PD-1) on T cells and of its ligand PD-L1 on cancer cells, inhibiting T cell activation [91]. At high concentrations, ROS may activate regulatory T cells with the ability to suppress the deleterious effect of an exacerbated cytotoxic T cell function. Therefore, while low levels of ROS are associated with a protective inflammatory state, high levels are associated with an anti-inflammatory immune signature, which appoints the targeting of ROS as a strategy to reduce Tregs activity and re-educate the immunosuppressive environment [90].

A recent clinical study in BC patients unveiled that high levels of ROS were associated with high intratumour heterogeneity, accompanied by infiltration of pro-inflammatory cells as M1-like macrophages, CD4⁺ T cells, CD8⁺ T cells, and B cells, but also of anti-inflammatory cells, as M2-like macrophages and Tregs. Interestingly, high ROS levels were significantly associated with worse OS in ER+/HER2- BC patients but not in the other subtypes [92]. Importantly, studies on the impact of oxidative stress on the immune system, treatment response, and prognosis of patients with HER2+ BC patients are lacking and constitute a relevant unmet need in the field.

7. Bioinformatic analysis of the redox-related gene signature of HER2+ BC

The specific mechanisms and proteins involved in cellular redox regulation relevant to HER2+ BC progression are not understood and

have not been thoroughly explored. Therefore, using the TCGA-BRCA dataset to examine HER2+ mRNA expression, we identified the redox-related signature of dysregulated genes in these tumours and their association with patient survival (Fig. 3). Despite the low number of samples included in the HER2+ subset of the TCGA-BRCA group, it was possible to identify 53 redox-related genes that fit these two criteria. For most genes identified (43 in 53), an increased expression was associated

with a higher HR, while the expression of only 10 genes was associated with a lower HR.

Of the 53 genes identified (Fig. 3) only a few have been experimentally shown to impact HER2+ BC. These include the Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit (EZH2), Mitogen Activated Protein Kinase 13 (MAPK13), NFE2 Like BZIP Transcription Factor 2 (NFE2L2), Histone Deacetylase 2 (HDAC2), Macrohistone 2A1

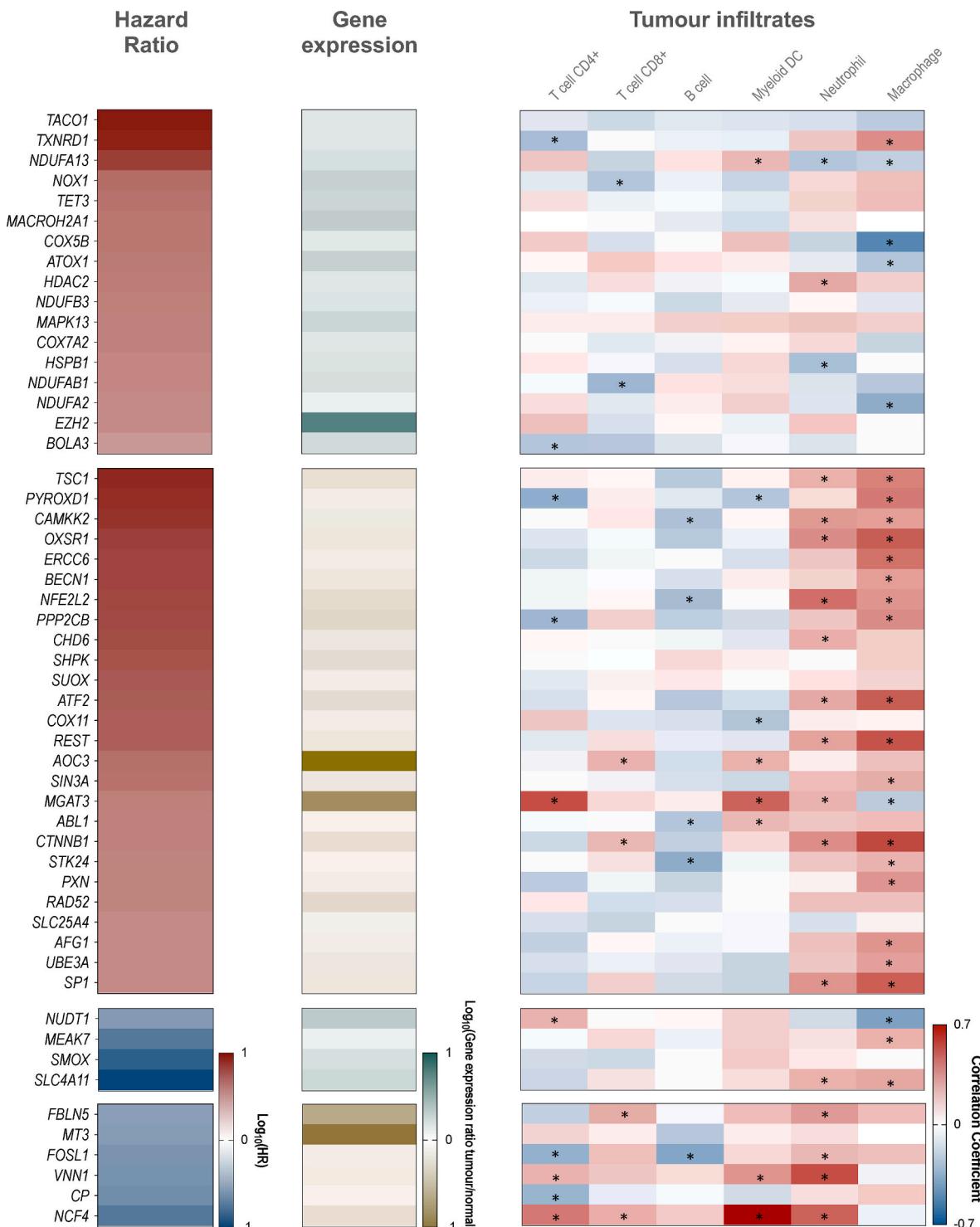


Fig. 3. Identification of genes related to redox homeostasis that are relevant for HER2+ BC. The list of 53 genes with expression dysregulation that are significantly associated with HER2+ BC patient survival is depicted. For each gene, the values corresponding to the Log10(HR), Log10(gene expression ratio tumour/normal tissue), and impact on the estimated abundance of different tumour infiltrates are depicted as heatmaps. * $p < 0.05$ from Spearman correlation.

(MACROH2A1), and the growth inhibitory protein tuberin (TSC2). Interestingly, EZH2 is strongly upregulated in HER2+ BC and is associated with lower patient survival. The overexpression of this gene is associated with a poor prognosis and a more aggressive HER2+ tumour behaviour. EZH2 activates the transcription of oestrogen receptor- α in HER2+ BC cells and has been implicated in promoting tumour growth, metastasis, and resistance to HER2-targeted therapies [93]. MAPK13 (also known as p38 δ) is upregulated in HER2+ BC and is associated with increased invasion, metastasis, and consequently with a lower survival rate [94]. NFE2L2 (also known as NRF2) is downregulated in HER2+ BC and is associated with a poorer outcome. This transcription factor is involved in cellular antioxidant response and survival signalling and

impacts several hallmarks of cancer [95]. Expression dysregulation of NFE2L2 was observed in HER2+ BC and leads to altered redox balance and increased oxidative stress [96]. HDAC2 is overexpressed and was previously implicated in HER2+ BC, where it was suggested as a potential therapeutic target given the association with an increased HR and because its inhibition can enhance the anti-tumour effects of HER2-targeted therapies [97]. MACROH2A1.2 promotes HER2 expression in BC cells [98]. Finally, loss of TSC2 function can lead to anti-HER2 therapy resistance [99].

Several redox-related genes identified here are strongly dysregulated and are associated with altered HR. Despite this, for many of these genes, few or no reports exist deciphering their involvement specifically

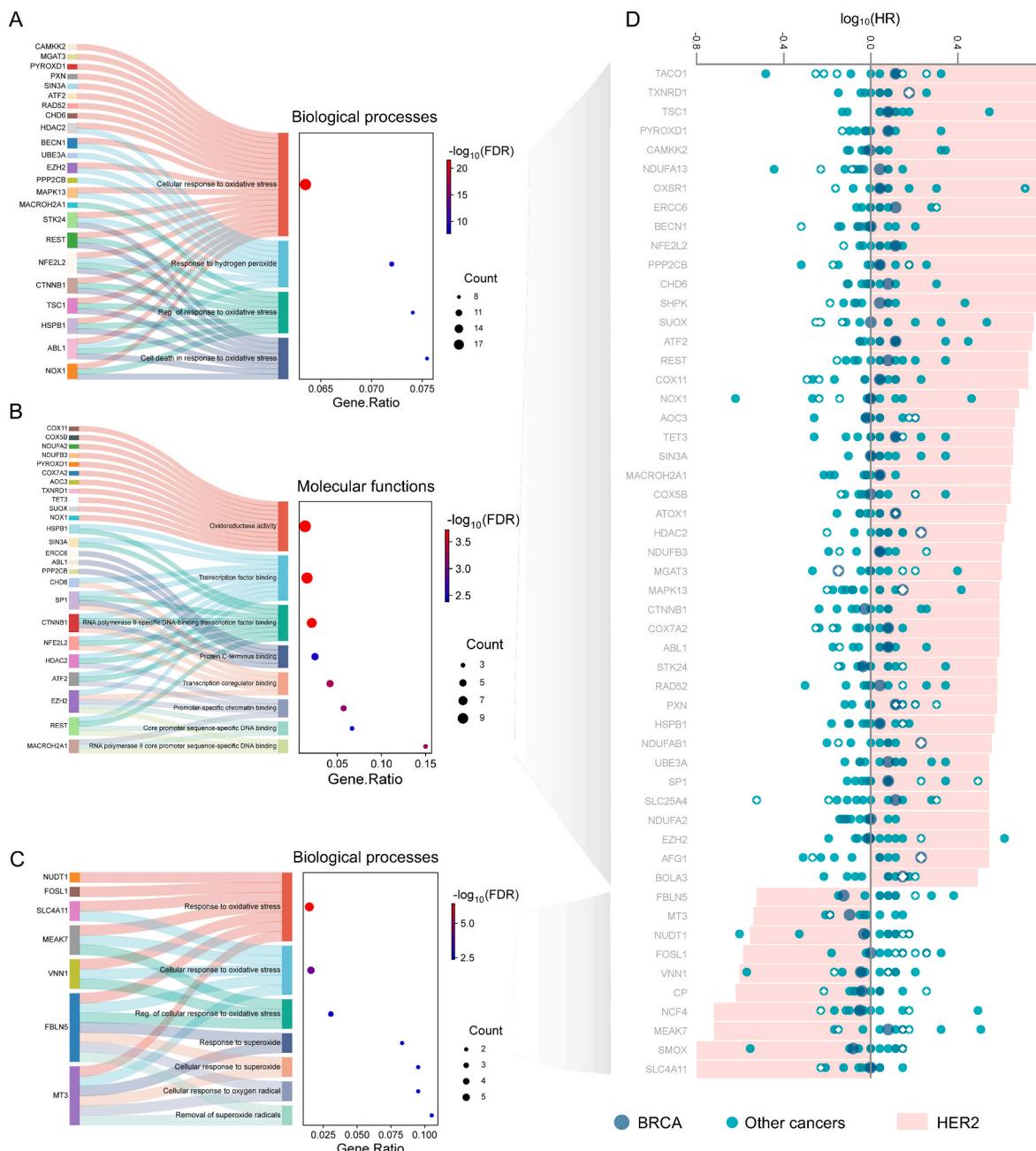


Fig. 4. Functions of the selected redox-related genes and association between their expression and cancer patient survival. (A–C) Gene enrichment analysis for biological processes and molecular function of genes associated with (A and B) higher or (C) lower HER2+ HR. (D) Overall patient survival is expressed as Log₁₀(Hazard Ratio) for each gene across the following cancer sites: breast, prostate, lung and bronchus, colon and rectum, skin melanoma, bladder, lymphoma, kidney, uterus, leukemia, pancreas, and thyroid. Pink bars represent HER2+ breast cancer Log₁₀(HR), and dots represent Log₁₀(HR) of each cancer group listed. White diamonds indicate statistical significance (Log-rank test, $p < 0.05$).

on HER2+ BC or the mechanisms underlying their putative effects. Examples of such genes include TACO1, PYROXD1, CAMKK2, AOC1, MGAT3, NUDT1, SLC4A11, TXNRD1, MT3 and NCF4.

Importantly, from the redox-related genes associated with high HR, 17 are highly expressed in HER2+ tumours, when compared to normal breast tissue, with no particular correlation with infiltration of specific subpopulations of immune cells. This is the case of TACO1, TXNRD1, NDUFA13, NOX1, TET3, MACROH2A1, COX5B, ATOX1, HDAC2, NDUFB3, MAPK13, COX7A2, HSPB1, NDUFAB1, NDUFA2, EZH2 and BOLA3. In opposite, 26 redox-related genes associated with high HR are downregulated in HER2+ tumours, in comparison to normal breast tissue, but are strongly correlated with enhanced infiltration of macrophages and neutrophils. This is the case for TSC1, PYROXD1, CAMKK2, OXSR1, ERCC6, BECN1, NFE2L2, PPP2CB, CHD6, SHPK, SUOX, ATF2, COX11, REST, AOC3, SIN3A, MGAT3, ABL1, CTNNB1, STK24, PXN, RAD52, SLC25A4, AFG1, UBE3A and SP1. From the 10 redox-related genes associated with a low HR, 4 are highly expressed in HER2+ tumours, in comparison to normal breast tissue, such as NUDT1, MEAK7, SMOX, SLC4A11 while 6 are downregulated in HER2+ tumours, in comparison to normal breast tissue, as FBLN5, MT3, FOSL1, VNN1, CP, and NCF4. All these genes associated with low HR in HER2+ breast tumours, are partially correlated with enhanced infiltration of macrophages, neutrophils, myeloid dendritic cells, and cytotoxic CD8⁺ T cells (Fig. 3).

To further explore the functions of redox-related genes associated with significant alterations in patient survival, we performed a gene enrichment analysis based on biological processes (Fig. 4A and C). The analysis revealed that many of these genes are linked to cellular responses to oxidative stress. Interestingly, genes associated with poorer prognosis (higher HR) were predominantly involved in the cellular response to hydrogen peroxide (Fig. 4A). Whereas those linked to more favourable outcomes were associated with the response to superoxide radicals (Fig. 4C), suggesting a differential impact of specific oxidative stress mediators on patient survival. For genes with higher HR values, a molecular function enrichment analysis identified two main functional groups (Fig. 4B). One group included genes encoding proteins with oxidoreductase activity, which are directly involved in redox homeostasis. The other group consisted of genes related to transcriptional regulation and DNA binding. Notably, there was considerable overlap between genes implicated in H₂O₂ response and those involved in transcriptional regulation, reinforcing the notion that H₂O₂ acts as a regulatory molecule, influencing gene expression patterns and cellular pathways.

8. Clinical applications

Research on prognostic genes in HER2+ breast cancer already plays a crucial role in clinical decision-making. The HER2DX test, which evaluates 27 genes, is a valuable tool for assessing prognosis in HER2-based chemotherapy and the likelihood of achieving a complete response to trastuzumab therapy. HER2DX can help identify candidates for escalation or de-escalation treatment strategies [100]. This underscores the importance of continuing prognostic gene research and the relevance of our study.

Considering the potential impact of redox regulation on HER2+ BC progression and therapy resistance, many of the genes listed in this work may prove valuable therapeutic targets or biomarkers and should therefore be further explored. Several of these genes are also associated with tumour progression events in other cancers. We compared the HR of redox-related gene expression in HER2+ BC with those calculated for other cancer types (Fig. 4D). Overall, the HR values suggest that the expression of this group of genes is not strongly associated with prognosis across all cancers. However, in diffuse large B-cell lymphoma, acute myeloid leukemia, and thyroid cancer, HR trends generally mirrored those observed in HER2+ BC (Supp. Fig. 1). Contrarily, kidney and colorectal cancers presented a distinct pattern. Notably, when

comparing HER2+ BC to overall breast cancer, the HR values for most genes followed similar directional trends, albeit with less pronounced variations. These findings highlight the distinct redox landscape of HER2+ BC and emphasize the necessity of tailoring approaches to each cancer subtype for more precise prognostic and therapeutic strategies.

Our interactions network analysis of proteins encoded by the identified redox-related genes revealed their involvement in three main biological processes: oxidative phosphorylation, response to oxidative stress, and regulation of transcription (Fig. 5A). Notably, many genes associated with response to oxidative stress are also involved in transcriptional control, suggesting that this response is, at least in part, mediated through transcriptional regulation. The Reactome pathways analysis (Fig. 5B) demonstrates that multiple proteins are implicated in four pathways known to play a role in cancer development. The Heme Oxygenase 1 (HMOX1) pathway may impact cell cycle and proliferation, as well as the immune TME [101]. Some genes implicated in the HMOX1 pathway are simultaneously involved in the TP53-mediated translational control. Consistently, it has been hypothesized that heme metabolism exerts a regulatory role of p53 expression to activate proliferation and cell survival signalling during carcinogenesis [101]. Not surprisingly, TP53-mediated translational control emerged as another pathway shared by some of the genes identified in this work. This transcription factor suppresses tumour growth through the regulation of several target genes with diverse biological functions [102]. One gene that may be regulated by TP53 is PTEN. Regulation of PTEN transcription also emerged in our Reactome analysis. PTEN is a key tumour suppression protein, and alterations in its status have been associated with tumorigenesis, progression, and therapy resistance in BC and particularly in HER2+ BC [103]. The fourth pathway shared by our selected genes is the VEGFA-VEGFR2 (vascular endothelial growth factor-A/vascular endothelial growth factor receptor 2), the most prominent ligand-receptor complex in the VEGF system. Signal transduction networks initiated by VEGFA/VEGFR2 lead to endothelial cell proliferation, migration, and survival, critical processes for angiogenesis and, therefore, cancer progression [104]. These findings highlight the intricate interplay between redox homeostasis and oncogenic signalling pathways.

9. Conclusion

TILs have increasingly gained attention as a valuable biomarker for predicting treatment response. ROS influence the TME, playing a fundamental role in the recruitment, survival, proliferation, and behaviour of immune cells. Nevertheless, the complex molecular mechanisms underlying the impact of redox and immune changes on the response to treatment and prognosis of HER2+ BC patients remain to be fully elucidated. Our analysis revealed that the altered expression of several redox-related genes could be associated with the prevalence of immune cell populations in the TME, and with patient survival. Our findings pave the way for future studies exploring new redox and immune-related biomarkers toward personalised medicine in HER2+ BC.

CRediT authorship contribution statement

Paulo Luz: Writing – original draft, Methodology, Investigation, Conceptualization. **Sofia Ramos:** Writing – original draft, Visualization, Software, Methodology, Investigation. **Maria José Oliveira:** Writing – review & editing, Formal analysis. **João G. Costa:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Nuno Saraiva:** Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation, Data curation, Conceptualization. **Ana S. Fernandes:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

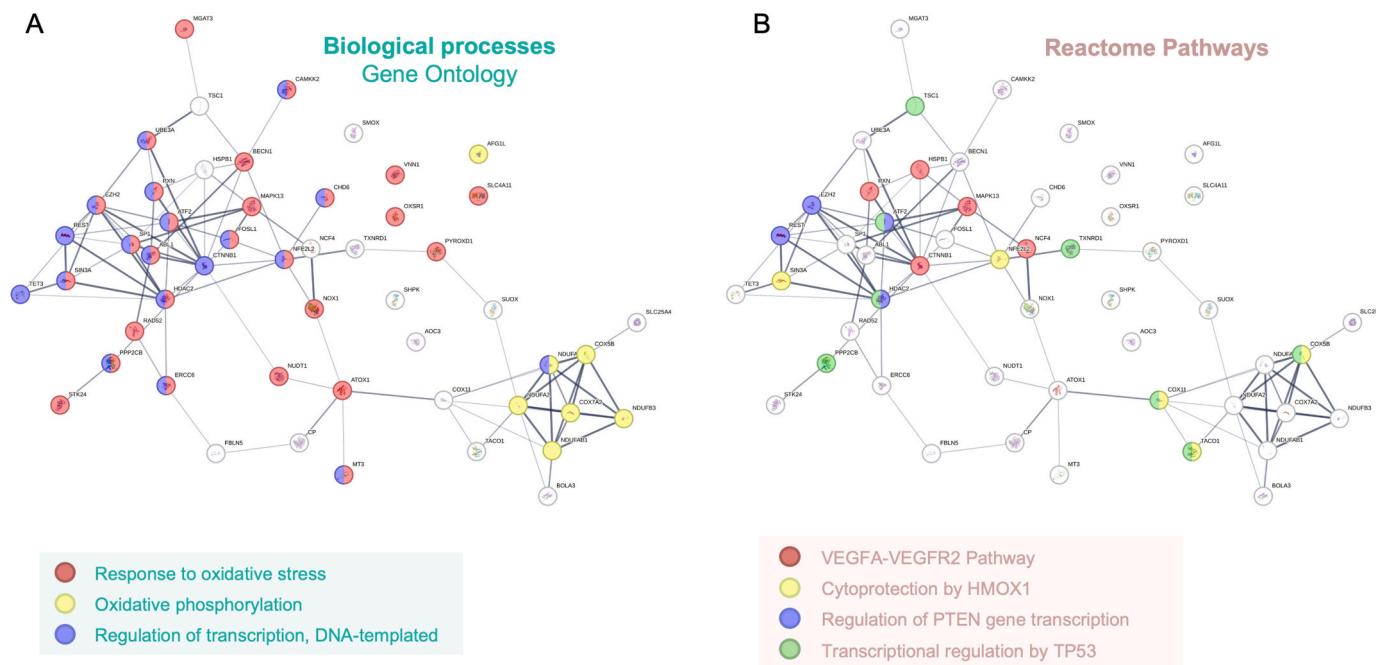


Fig. 5. Known and predicted protein–protein interactions network of proteins encoded by the selected genes. The most relevant, comprehensive and enriched (A) Biological processes and (B) Reactome Pathways are highlighting in colour. PPI enrichment p-value < 5e-14.

Ethical approval

No ethical approval was required.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used ChatGPT 4.0 in order to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.redox.2025.103609>.

Data availability

The article used the TCGA dataset, which is publicly available.

References

- M.T. van Mackelenbergh, S. Loibl, M. Untch, M. Buyse, C.E. Geyer Jr., L. Gianni, A. Schneeweiss, P. Conte, M. Piccart, H. Bonnefoi, C. Jackisch, V. Nekljudova, G. Tang, P. Valagussa, C. Neate, R. Gelber, C. Poncet, P. Squifflet, E.D. Saad, D. Heinzmann, CTNeoBC project, Pathologic complete response and individual patient prognosis after neoadjuvant chemotherapy plus anti-human epidermal growth factor receptor 2 therapy of human epidermal growth factor receptor 2-positive early breast cancer, *J. Clin. Oncol. : Offic. J. Am. Soc. Clin. Oncol.* 41 (16) (2013) 2998–3008, <https://doi.org/10.1200/JCO.22.02241>.
- P. Cortazar, L. Zhang, M. Untch, K. Mehta, J.P. Costantino, N. Wolmark, H. Bonnefoi, D. Cameron, L. Gianni, P. Valagussa, S.M. Swain, T. Prowell, S. Loibl, D.L. Wickerham, J. Bogaerts, J. Baselga, C. Perou, G. Blumenthal, J. Blohmer, E.P. Mamounas, G. von Minckwitz, Pathological complete response and long-term clinical benefit in breast cancer: the CTNeoBC pooled analysis, *Lancet (London, England)* 384 (9938) (2014) 164–172, [https://doi.org/10.1016/S0140-6736\(13\)62422-8](https://doi.org/10.1016/S0140-6736(13)62422-8).
- S. Escrivá-de-Romaní, M. Arumí, E. Zamora, M. Bellet, Neadjuvant model as a platform for research in breast cancer and novel targets under development in this field, *Breast Care* 13 (4) (2018) 251–262, <https://doi.org/10.1159/000492122>.
- M.G. Davey, F. Browne, N. Miller, A.J. Lowery, M.J. Kerin, Pathological complete response as a surrogate to improved survival in human epidermal growth factor receptor-2-positive breast cancer: systematic review and meta-analysis, *BJS open* 6 (3) (2022) zrac028, <https://doi.org/10.1093/bjsopen/zrac028>.
- S. Verma, D. Miles, L. Gianni, I.E. Krop, M. Welslau, J. Baselga, M. Pegram, D. Y. Oh, V. Diéras, E. Guardino, L. Fang, M.W. Lu, S. Olsen, K. Blackwell, EMILIA Study Group, Trastuzumab emtansine for HER2-positive advanced breast cancer, *N. Engl. J. Med.* 367 (19) (2012) 1783–1791, <https://doi.org/10.1056/NEJMoa1209124>.
- J. Cortés, S.B. Kim, W.P. Chung, S.A. Im, Y.H. Park, R. Hegg, M.H. Kim, L. M. Tseng, V. Petry, C.F. Chung, H. Iwata, E. Hamilton, G. Curigliano, B. Xu, C. S. Huang, J.H. Kim, J.W.Y. Chiu, J.L. Pedrin, C. Lee, Y. Liu, DESTINY-Breast03 Trial Investigators, Trastuzumab deruxtecan versus trastuzumab emtansine for breast cancer, *N. Engl. J. Med.* 386 (12) (2022) 1143–1154, <https://doi.org/10.1056/NEJMoa2115022>.
- F. Cardoso, S. Paluch-Shimon, E. Schumacher-Wulf, L. Matos, K. Gelmon, M. S. Aapro, J. Bajpai, C.H. Barrios, J. Bergh, E. Bergsten-Nordström, L. Biganzoli, M. J. Cardoso, L.A. Carey, M. Chavez-MacGregor, R. Chidebe, J. Cortés, G. Curigliano, R.A. Dent, N.S. El Saghir, A. Eniu, E.P. Winer, 6th and 7th International consensus guidelines for the management of advanced breast cancer (ABC guidelines 6 and 7), *Breast (Edinburgh, Scotland)* 76 (2024) 103756, <https://doi.org/10.1016/j.breast.2024.103756>.
- S.E. Stanton, S. Adams, M.L. Disis, Variation in the incidence and magnitude of tumour-infiltrating lymphocytes in breast cancer subtypes: a systematic review, *JAMA Oncol.* 2 (10) (2016) 1354–1360, <https://doi.org/10.1001/jamaoncol.2016.1061>.
- R. Malla, N. Surepalli, B. Farran, S.V. Malhotra, G.P. Nagaraju, Reactive oxygen species (ROS): critical roles in breast tumour microenvironment, *Crit. Rev. Oncol.-Hematol.* 160 (2021) 103285, <https://doi.org/10.1016/j.critrevonc.2021.103285>.
- M. Azmanova, A. Pittó-Barry, Oxidative stress in cancer therapy: friend or enemy? *Chembiochem : Euro. J. Chem. Biol.* 23 (10) (2022) e202100641 <https://doi.org/10.1002/cbic.202100641>.

- [11] Z. Tang, B. Kang, C. Li, T. Chen, Z. Zhang, GEPIA2: an enhanced web server for large-scale expression profiling and interactive analysis, Nucleic Acids Res. 47 (W1) (2019) W556–W560, <https://doi.org/10.1093/nar/gkz430>.
- [12] D.S. Chandrashekhar, B. Bashel, S.A.H. Balasubramanya, C.J. Creighton, I. Ponce-Rodriguez, B.V.S.K. Chakravarthi, S. Varambally, UALCAN: a portal for facilitating tumour subgroup gene expression and survival analyses, Neoplasia 19 (8) (2017) 649–658, <https://doi.org/10.1016/j.neo.2017.05.002>.
- [13] T. Li, J. Fu, Z. Zeng, D. Cohen, J. Li, Q. Chen, B. Li, X.S. Liu, TIMER2.0 for analysis of tumour-infiltrating immune cells, Nucleic Acids Res. 48 (W1) (2020) W509–W514, <https://doi.org/10.1093/nar/gkaa407>.
- [14] B. Li, E. Severson, J.C. Pignon, H. Zhao, T. Li, J. Novak, P. Jiang, H. Shen, J. C. Aster, S. Rodig, S. Signoretti, J.S. Liu, X.S. Liu, Comprehensive analyses of tumour immunity: implications for cancer immunotherapy, Genome Biol. 17 (1) (2016) 174, <https://doi.org/10.1186/s13059-016-1028-7>.
- [15] R.L. Siegel, A.N. Giaquinto, A. Jemal, Cancer statistics, 2024, CA Cancer J. Clin. 74 (1) (2024) 12–49, <https://doi.org/10.3322/caac.21820>.
- [16] S.X. Ge, D. Jung, R. Yao, ShinyGO: a graphical gene-set enrichment tool for animals and plants, Bioinformatics (Oxford, England) 36 (8) (2020) 2628–2629, <https://doi.org/10.1093/bioinformatics/btz931>.
- [17] D. Tang, M. Chen, X. Huang, G. Zhang, L. Zeng, G. Zhang, S. Wu, Y. Wang, SRplot: a free online platform for data visualization and graphing, PLoS One 18 (11) (2023) e0294236, <https://doi.org/10.1371/journal.pone.0294236>.
- [18] D.F. Hayes, HER2 and breast cancer - a phenomenal success story, N. Engl. J. Med. 381 (13) (2019) 1284–1286, <https://doi.org/10.1056/NEJMcb1909386>.
- [19] X. Cheng, A comprehensive review of HER2 in cancer biology and therapeutics, Genes 15 (7) (2024) 903, <https://doi.org/10.3390/genes15070903>.
- [20] D. Graus-Porta, R.R. Beerli, J.M. Daly, N.E. Hynes, ErBB-2, the preferred heterodimerization partner of all ErbB receptors, is a mediator of lateral signaling, EMBO J. 16 (7) (1997) 1647–1655, <https://doi.org/10.1093/emboj/16.7.1647>.
- [21] M.A. Olayioye, R.M. Neve, H.A. Lane, N.E. Hynes, The ErbB signaling network: receptor heterodimerization in development and cancer, EMBO J. 19 (13) (2000) 3159–3167, <https://doi.org/10.1093/emboj/19.13.3159>.
- [22] R. Falcioni, A. Antonini, P. Nisticò, S. Di Stefano, M. Crescenzi, P.G. Natali, A. Sacchi, Alpha 6 beta 4 and alpha 6 beta 1 integrins associate with ErbB-2 in human carcinoma cell lines, Exp. Cell Res. 236 (1) (1997) 76–85, <https://doi.org/10.1006/excr.1997.3695>.
- [23] H. Khouri, M.A. Naujokas, D. Zuo, V. Sangwan, M.M. Frigault, S. Petkiewicz, D. L. Dankort, W.J. Muller, M. Park, HGF converts ErbB2/Neu epithelial morphogenesis to cell invasion, Mol. Biol. Cell 16 (2) (2005) 550–561, <https://doi.org/10.1091/mbc.e04-07-0567>.
- [24] P. Arora, B.D. Cuevas, A. Russo, G.L. Johnson, J. Trejo, Persistent transactivation of EGFR and ErbB2/HER2 by protease-activated receptor-1 promotes breast carcinoma cell invasion, Oncogene 27 (32) (2008) 4434–4445, <https://doi.org/10.1038/onc.2008.84>.
- [25] J. Wang, B. Xu, Targeted therapeutic options and future perspectives for HER2-positive breast cancer, Signal Transduct. Targeted Ther. 4 (2019) 34, <https://doi.org/10.1038/s41392-019-0069-2>.
- [26] D.J. Slamon, B. Leyland-Jones, S. Shak, H. Fuchs, V. Paton, A. Bajamonde, T. Fleming, W. Eiermann, J. Wolter, M. Pegram, J. Baselga, L. Norton, Use of chemotherapy plus monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2, N. Engl. J. Med. 344 (11) (2001) 783–792, <https://doi.org/10.1056/NEJM200103153441101>.
- [27] H.S. Cho, K. Mason, K.X. Ramy, A.M. Stanley, S.B. Gabelli, D.W. Denney Jr., D. J. Leahy, Structure of the extracellular region of HER2 alone and in complex with the Herceptin Fab, Nature 421 (6924) (2003) 756–760, <https://doi.org/10.1038/nature01392>.
- [28] A.J. Genuino, U. Chaikledkaew, D.O. The, T. Reungwetwattana, A. Thakinstian, Adjuvant trastuzumab regimen for HER2-positive early-stage breast cancer: a systematic review and meta-analysis, Expert Rev. Clin. Pharmacol. 12 (8) (2019) 815–824, <https://doi.org/10.1080/17512433.2019.1637252>.
- [29] H. Maadi, M.H. Soheilifar, W.S. Choi, A. Moshtaghian, Z. Wang, Trastuzumab mechanism of action; 20 Years of research to unravel a dilemma, Cancers 13 (14) (2021) 3540, <https://doi.org/10.3390/cancers13143540>.
- [30] L. Arnould, M. Gelly, F. Penault-Llorca, L. Benoit, F. Bonnetaïn, C. Migeon, V. Cabaret, V. Fermeaux, P. Bertheau, J. Garnier, J.F. Jeannin, B. Couder, Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? Br. J. Cancer 94 (2) (2006) 259–267, <https://doi.org/10.1038/sj.bjc.6602930>.
- [31] S. Park, Z. Jiang, E.D. Mortenson, L. Deng, O. Radkevich-Brown, X. Yang, H. Sattar, Y. Wang, N.K. Brown, M. Greene, Y. Liu, J. Tang, S. Wang, Y.X. Fu, The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity, Cancer Cell 18 (2) (2010) 160–170, <https://doi.org/10.1016/j.ccr.2010.06.014>.
- [32] S. Loibl, F. André, T. Bacheler, C.H. Barrios, J. Bergh, H.J. Burstein, M.J. Cardoso, L.A. Carey, S. Dawood, L. Del Mastro, C. Denkert, E.M. Fallenberg, P.A. Francis, H. Gamal-Eldin, K. Gelmon, C.E. Geyer, M. Gnant, V. Guarneri, S. Gupta, S. B. Kim, ESMO Guidelines Committee. Electronic address: clinicalguidelines@esmo.org, Early breast cancer: ESMO Clinical Practice Guideline for diagnosis, treatment and follow-up, Ann. Oncol. : Offic. J. Euro. Soc. Med. Oncol. 35 (2) (2024) 159–182, <https://doi.org/10.1016/j.annonc.2023.11.016>.
- [33] F.J. Esteva, E. Katz, Tailoring neoadjuvant therapy in human epidermal growth factor receptor 2-positive early breast cancer: recent advances and strategies, JCO Oncol. Pract. 20 (8) (2024) 1046–1054, <https://doi.org/10.1200/OP.23.00563>.
- [34] S. Loibl, C.S. Huang, M.S. Mano, E.P. Mamounas, C.E. Geyer Jr., M. Untch, J. C. Thery, I. Schwaner, S. Limentani, N. Loman, K. Lübbe, J.C. Chang, T. Hatschek, D. Tesarowski, C. Song, S. Lysbet de Haas, T. Boulet, C. Lambertini, N. Wolmark, Adjuvant trastuzumab emtansine in HER2-positive breast cancer patients with HER2-negative residual invasive disease in KATHERINE, NPJ breast cancer 8 (1) (2022) 106, <https://doi.org/10.1038/s41523-022-00477-z>.
- [35] G. von Minckwitz, M. Procter, E. de Azambuja, D. Zardavas, M. Benyunes, G. Viale, T. Suter, A. Arahmani, N. Rouchet, E. Clark, A. Knott, I. Lang, C. Levy, D. A. Yardley, J. Bines, R.D. Gelber, M. Piccart, J. Baselga, APHINITY Steering Committee and Investigators, Adjuvant pertuzumab and trastuzumab in early HER2-positive breast cancer, N. Engl. J. Med. 377 (2) (2017) 122–131, <https://doi.org/10.1056/NEJMoa1703643>.
- [36] K. Berns, H.M. Horlings, B.T. Hennessy, M. Madiredjo, E.M. Hijmans, K. Beelen, S. C. Linn, A.M. Gonzalez-Angulo, K. Stemke-Hale, M. Hauptmann, R. L. Beijersbergen, G.B. Mills, M.J. van de Vijver, R. Bernards, A functional genetic approach identifies the PI3K pathway as a major determinant of trastuzumab resistance in breast cancer, Cancer Cell 12 (4) (2007) 395–402, <https://doi.org/10.1016/j.ccr.2007.08.030>.
- [37] V. Serra, B. Markman, M. Scaltriti, P.J. Eichhorn, V. Valero, M. Guzman, M. L. Botero, E. Llonch, F. Atzori, S. Di Cosimo, M. Maira, C. Garcia-Echeverria, J. L. Parra, J. Arribas, J. Baselga, NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations, Cancer Res. 68 (19) (2008) 8022–8030, <https://doi.org/10.1158/0008-5472.CAN-08-1385>.
- [38] M. Scaltriti, F. Rojo, A. Ocaña, J. Anido, M. Guzman, J. Cortes, S. Di Cosimo, X. Matias-Guiu, S. Ramon y Cajal, J. Arribas, J. Baselga, Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer, J. Natl. Cancer Inst. 99 (8) (2007) 628–638, <https://doi.org/10.1093/jnci/djk134>.
- [39] G.K. Scott, R. Robles, J.W. Park, P.A. Montgomery, J. Daniel, W.E. Holmes, J. Lee, G.A. Keller, W.L. Li, B.M. Fendly, A truncated intracellular HER2/neu receptor produced by alternative RNA processing affects growth of human carcinoma cells, Mol. Cell Biol. 13 (4) (1993) 2247–2257, <https://doi.org/10.1128/mcb.13.4.2247-2257.1993>.
- [40] Y. Lu, X. Zi, Y. Zhao, D. Mascarenhas, M. Pollak, Insulin-like growth factor-I receptor signaling and resistance to trastuzumab (Herceptin), J. Natl. Cancer Inst. 93 (24) (2001) 1852–1857, <https://doi.org/10.1093/jnci/93.24.1852>.
- [41] S. Boero, A. Morabito, B. Banelli, B. Cardinali, B. Dozin, G. Lunardi, P. Piccioli, S. Lastraioli, R. Carosio, S. Salvi, A. Levaggi, F. Poggio, A. D'Alonzo, M. Romani, L. Del Mastro, A. Poggi, M.P. Pistillo, Analysis of in vitro ADCC and clinical response to trastuzumab: possible relevance of FcγRIIIA/FcγRIIA gene polymorphisms and HER-2 expression levels on breast cancer cell lines, J. Transl. Med. 13 (2015) 324, <https://doi.org/10.1186/s12967-015-0680-0>.
- [42] D.J. Fassler, L.A. Torre-Healy, R. Gupta, A.M. Hamilton, S. Kobayashi, S.C. Van Alsten, Y. Zhang, T. Kurc, R.A. Moffitt, M.A. Troester, K.A. Hoadley, J. Saltz, Spatial characterization of tumour-infiltrating lymphocytes and breast cancer progression, Cancers 14 (9) (2022 Apr 26) 2148, <https://doi.org/10.3390/cancers14092148>. PMID: 35565277; PMCID: PMC9105398.
- [43] A. Andersson, L. Larsson, L. Stenbeck, F. Salmén, A. Ehinger, S.Z. Wu, G. Al-Eryani, D. Roden, A. Swarbrick, Å. Borg, J. Frisén, C. Engblom, J. Lundeberg, Spatial deconvolution of HER2-positive breast cancer delineates tumour-associated cell type interactions, Nat. Commun. 12 (1) (2021) 6012, <https://doi.org/10.1038/s41467-021-26271-2>.
- [44] S. Ganjoo, P. Gupta, H.I. Corbali, S. Nanez, T.S. Riad, L.K. Duong, H. B. Barsoumian, F. Masrourpour, H. Jiang, J.W. Welsh, M.A. Cortez, The role of tumour metabolism in modulating T-Cell activity and in optimizing immunotherapy, Front. Immunol. 14 (2023) 1172931, <https://doi.org/10.3389/fimmu.2023.1172931>.
- [45] X. Du, Z. Zhou, Y. Shao, K. Qian, Y. Wu, J. Zhang, M. Cui, J. Wang, S. Wang, Y. Tai, Immunoarchitectural patterns as potential prognostic factors for invasive ductal breast cancer, NPJ breast cancer 8 (1) (2022) 26, <https://doi.org/10.1038/s41523-022-00389-y>.
- [46] C. Denkert, S. Loibl, A. Noske, M. Roller, B.M. Müller, M. Komor, J. Budczies, S. Darb-Esfahani, R. Kronenwett, C. Hanusch, C. von Törne, W. Weichert, K. Engels, C. Solbach, I. Schrader, M. Dietel, G. von Minckwitz, Tumour-associated lymphocytes as an independent predictor of response to neoadjuvant chemotherapy in breast cancer, J. Clin. Oncol. : Offic. J. Am. Soc. Clin. Oncol. 28 (1) (2010) 105–113, <https://doi.org/10.1200/JCO.2009.23.7370>.
- [47] C. Denkert, G. von Minckwitz, J.C. Bräse, B.V. Sinn, S. Gade, R. Kronenwett, B. M. Pfitzner, C. Salat, S. Loi, W.D. Schmitt, C. Schem, K. Fisch, S. Darb-Esfahani, K. Mehta, C. Sotiriou, S. Wienert, P. Klare, F. André, F. Klauschen, J.U. Blohmer, S. Loibl, Tumour-infiltrating lymphocytes and response to neoadjuvant chemotherapy with or without carboplatin in human epidermal growth factor receptor 2-positive and triple-negative primary breast cancers, J. Clin. Oncol. : Offic. J. Am. Soc. Clin. Oncol. 33 (9) (2015) 983–991, <https://doi.org/10.1200/JCO.2014.58.1967>.
- [48] R.K. Hills, R. Bradley, J. Braybrooke, R.G. Gray, H. Taylor, C. Denkert, S.S. Badve, R.S. Kim, M. Lacroix-Triki, M.M. Regan, D.F. Hayes, M. Dowsett, A.N.J. Tutt, R. D. Gelber, D.A. Cameron, J.C.S. Bergh, S.M. Swain, S. Michiels, S. Loi, R. Salgado, Do tumour infiltrating lymphocytes (TILs) predict benefits from trastuzumab therapy for HER2 positive breast cancer? Meta-analysis of individual patient data from 4097 women in 5 trials, J. Clin. Oncol. 41 (16_suppl) (2023), https://doi.org/10.1200/JCO.2023.41.16_suppl.508.
- [49] S.M. Mahmoud, E.C. Paish, D.G. Powe, R.D. Macmillan, M.J. Grainge, A.H. Lee, I. O. Ellis, A.R. Green, Tumour-infiltrating CD8+ lymphocytes predict clinical outcome in breast cancer, J. Clin. Oncol. : Offic. J. Am. Soc. Clin. Oncol. 29 (15) (2011) 1949–1955, <https://doi.org/10.1200/JCO.2010.30.5037>.

- [50] S. Ladoire, G. Mignot, S. Dabakuyo, L. Arnould, L. Apetoh, C. Rébé, B. Couder, F. Martin, M.H. Bizollon, A. Vanoli, C. Coutant, P. Fumoleau, F. Bonnetain, F. Ghiringhelli, In situ immune response after neoadjuvant chemotherapy for breast cancer predicts survival, *J. Pathol.* 224 (3) (2011) 389–400, <https://doi.org/10.1002/path.2866>.
- [51] M.V. Dieci, N. Radosevic-Robin, S. Fineberg, G. van den Eynden, N. Ternes, et al., Update on tumour-infiltrating lymphocytes (TILs) in breast cancer, including recommendations to assess TILs in residual disease after neoadjuvant therapy and in carcinoma in situ: a report of the International Immuno-Oncology Biomarker Working Group on Breast Cancer, *Semin. Cancer Biol.* 52 (Pt 2) (2018 Oct) 16–25, <https://doi.org/10.1016/j.semcancer.2017.10.003>. Epub 2017 Oct 9. Review. PubMed PMID: 29024776.
- [52] S. Kurozumi, K. Inoue, H. Matsumoto, T. Fujii, J. Horiguchi, T. Oyama, M. Kurosumi, K. Shirabe, Prognostic utility of tumour-infiltrating lymphocytes in residual tumour after neoadjuvant chemotherapy with trastuzumab for HER2-positive breast cancer, *Sci. Rep.* 9 (1) (2019) 1583, <https://doi.org/10.1038/s41598-018-38272-1>.
- [53] A.S. Hamy, J.Y. Pierga, A. Sabaila, E. Laas, H. Bonsang-Kitzis, C. Laurent, A. Vincent-Salomon, P. Cottu, F. Lerebours, R. Rouzier, M. Lae, F. Reynal, Stromal lymphocyte infiltration after neoadjuvant chemotherapy is associated with aggressive residual disease and lower disease-free survival in HER2-positive breast cancer, *Ann. Oncol.* : Offic. J. Euro. Soc. Med. Oncol. 28 (9) (2017) 2233–2240, <https://doi.org/10.1093/annonc/mdx309>.
- [54] F. Miglietta, M. Ragazzi, B. Fernandes, G. Griguolo, D. Massa, F. Girardi, M. Bottosso, A. Bisagni, G. Zarrilli, F. Porra, D. Iannaccone, L. Dore, M. Gaudio, G. Santandrea, M. Fassan, M. Lo Mele, R. De Sanctis, A. Zambelli, G. Bisagni, V. Guarneri, M.V. Dieci, A prognostic model based on residual cancer burden and tumour-infiltrating lymphocytes on residual disease after neoadjuvant therapy in HER2+ breast cancer, *Clin. Cancer Res.* : Offic. J. Am. Assoc. Cancer Res. 29 (17) (2023) 3429–3437, <https://doi.org/10.1158/1078-0432.CCR-23-0480>.
- [55] G. Griguolo, G. Serna, T. Pascual, R. Fasani, X. Guardia, N. Chic, L. Paré, S. Pernas, M. Muñoz, M. Oliveira, M. Vidal, A. Llombart-Cussac, J. Cortés, P. Galván, B. Bermejo, N. Martínez, R. López, S. Morales, I. Garau, L. Manso, P. Nuciforo, Immune microenvironment characterisation and dynamics during anti-HER2-based neoadjuvant treatment in HER2-positive breast cancer, *npj Precis. Oncol.* 5 (1) (2021) 23, <https://doi.org/10.1038/s41698-021-00163-6>.
- [56] P. Luz, I. Fernandes, J. Magalhães, R.T. Sousa, P. Faísca, J.G. Costa, A. S. Fernandes, Tumour-infiltrating lymphocytes in early breast cancer: an exploratory analysis focused on HER2+ subtype in Portuguese patients, *Curr. Med. Res. Opin.* 38 (8) (2022) 1379–1382, <https://doi.org/10.1080/03007995.2022.2096334>.
- [57] H.J. Kim, H. Cantor, CD4 T-cell subsets and tumour immunity: the helpful and the not-so-helpful, *Cancer Immunol. Res.* 2 (2) (2014) 91–98, <https://doi.org/10.1158/2326-6066.CIR-13-0216>.
- [58] M. Schmidt, V. Weyer-Eberl, J.G. Hengstler, A.S. Heimes, K. Almstedt, A. Gerhold-Ay, A. Lebrecht, M.J. Battista, A. Hasenburg, U. Sahin, K.T. Kalogeras, P.L. Kellokumpu-Lehtinen, G. Fountzilas, R.M. Wirtz, H. Joensuu, Prognostic impact of CD4-positive T cell subsets in early breast cancer: a study based on the FinHer trial patient population, *Breast Cancer Res.* 20 (1) (2018) 15, <https://doi.org/10.1186/s13058-018-0942-x>.
- [59] S.M. Mahmoud, E.C. Paish, D.G. Powe, R.D. Macmillan, A.H. Lee, I.O. Ellis, A. R. Green, An evaluation of the clinical significance of FOXP3+ infiltrating cells in human breast cancer, *Breast Cancer Res. Treat.* 127 (1) (2011) 99–108, <https://doi.org/10.1007/s10549-010-0987-8>.
- [60] G.J. Bates, S.B. Fox, C. Han, R.D. Leek, J.F. Garcia, A.L. Harris, A.H. Banham, Quantification of regulatory T cells enables the identification of high-risk breast cancer patients and those at risk of late relapse, *J. Clin. Oncol.* : Offic. J. Am. Soc. Clin. Oncol. 24 (34) (2006) 5373–5380, <https://doi.org/10.1200/JCO.2006.05.9584>.
- [61] L. Arnould, M. Gelly, F. Penault-Llorca, L. Benoit, F. Bonnetain, C. Migeon, V. Cabaret, V. Fermeaux, P. Bertheau, J. Garnier, J.F. Jeannin, B. Couder, Trastuzumab-based treatment of HER2-positive breast cancer: an antibody-dependent cellular cytotoxicity mechanism? *Br. J. Cancer* 94 (2) (2006) 259–267, <https://doi.org/10.1038/sj.bjc.6602930>.
- [62] S. Park, Z. Jiang, E.D. Mortenson, L. Deng, O. Radkevich-Brown, X. Yang, H. Sattar, Y. Wang, N.K. Brown, M. Greene, Y. Liu, J. Tang, S. Wang, Y.X. Fu, The therapeutic effect of anti-HER2/neu antibody depends on both innate and adaptive immunity, *Cancer Cell* 18 (2) (2010) 160–170, <https://doi.org/10.1016/j.ccr.2010.06.014>.
- [63] K. El-Sahwi, S. Bellone, E. Cocco, M. Cargnelutti, F. Casagrande, M. Bellone, M. Abu-Khalaf, N. Buza, F.A. Tavassoli, P. Hui, D.A. Silasi, M. Azodi, P. E. Schwartz, T.J. Rutherford, S. Pecorelli, A.D. Santin, In vitro activity of pertuzumab in combination with trastuzumab in uterine serous papillary adenocarcinoma, *Br. J. Cancer* 102 (1) (2010) 134–143, <https://doi.org/10.1038/sj.bjc.6605448>.
- [64] G. Tóth, A. Szőr, L. Simon, Y. Yarden, J. Szöllösi, G. Vereb, The combination of trastuzumab and pertuzumab administered at approved doses may delay development of trastuzumab resistance by additively enhancing antibody-dependent cell-mediated cytotoxicity, *mAbs* 8 (7) (2016) 1361–1370, <https://doi.org/10.1080/19420862.2016.1204503>.
- [65] A. Muntasell, M. Cabo, S. Servitja, I. Tusquets, M. Martínez-García, A. Rovira, F. Rojo, J. Albanell, M. López-Botet, Interplay between natural killer cells and anti-HER2 antibodies: perspectives for breast cancer immunotherapy, *Front. Immunol.* 8 (2017) 1544, <https://doi.org/10.3389/fimmu.2017.01544>.
- [66] L. Gianni, G. Bianchini, P. Valagussa, A. Belousov, M. Thomas, G. Ross, L. Pusztai, Adaptive immune system and immune checkpoints are associated with response to pertuzumab (P) and trastuzumab (H) in the NeoSphere study, *Cancer Res.* 72 (24 Supplement) (2012) S6–S7, <https://doi.org/10.1158/0008-5472.SABCS12-S6-7>.
- [67] S.J. Luen, R. Salgado, S. Fox, P. Savas, J. Eng-Wong, E. Clark, A. Kiermaier, S. M. Swain, J. Baselga, S. Michiels, S. Loi, Tumour-infiltrating lymphocytes in advanced HER2-positive breast cancer treated with pertuzumab or placebo in addition to trastuzumab and docetaxel: a retrospective analysis of the CLEOPATRA study, *Lancet Oncol.* 18 (1) (2017) 52–62, [https://doi.org/10.1016/S1470-2045\(16\)30631-3](https://doi.org/10.1016/S1470-2045(16)30631-3).
- [68] S. Liu, B. Chen, S. Burugu, S. Leung, D. Gao, S. Virk, Z. Kos, W.R. Parulekar, L. Shepherd, K.A. Gelmon, T.O. Nielsen, Role of cytotoxic tumour-infiltrating lymphocytes in predicting outcomes in metastatic HER2-positive breast cancer: a secondary analysis of a randomized clinical trial, *JAMA Oncol.* 3 (11) (2017) e172085, <https://doi.org/10.1001/jamaoncol.2017.2085>.
- [69] H. Sies, Oxidative stress: a concept in redox biology and medicine, *Redox Biol.* 4 (2015) 180–183, <https://doi.org/10.1016/j.redox.2015.01.002>.
- [70] J.D. Hayes, A.T. Dinkova-Kostova, K.D. Tew, Oxidative stress in cancer, *Cancer Cell* 38 (2) (2020) 167–197, <https://doi.org/10.1016/j.ccr.2020.06.001>.
- [71] H. Sies, D.P. Jones, Reactive oxygen species (ROS) as pleiotropic physiological signalling agents, *Nat. Rev. Mol. Cell Biol.* 21 (7) (2020) 363–383, <https://doi.org/10.1038/s41580-020-0230-3>.
- [72] M. Azmanova, A. Pitti-Barry, Oxidative stress in cancer therapy: friend or enemy? *ChemBioChem : Euro. J. Chem. Biol.* 23 (10) (2022) e202100641 <https://doi.org/10.1002/cbic.202100641>.
- [73] E.C. Cheung, K.H. Vousden, The role of ROS in tumour development and progression, *Nat. Rev. Cancer* 22 (5) (2022) 280–297, <https://doi.org/10.1038/s41568-021-00435-0>.
- [74] V. Sosa, T. Moliné, R. Somoza, R. Paciucci, H. Kondoh, M.E. Leonart, Oxidative stress and cancer: an overview, *Ageing Res. Rev.* 12 (1) (2013) 376–390, <https://doi.org/10.1016/j.arr.2012.10.004>.
- [75] A. Mencalha, V.J. Victorino, R. Cecchini, C. Panis, Mapping oxidative changes in breast cancer: understanding the basic to reach the clinics, *Anticancer Res.* 34 (3) (2014) 1127–1140.
- [76] E. Panieri, M.M. Santoro, ROS homeostasis and metabolism: a dangerous liaison in cancer cells, *Cell Death Dis.* 7 (6) (2016) e2253, <https://doi.org/10.1038/cddis.2016.105>.
- [77] V.J. Victorino, L. Pizzatti, P. Michelletti, C. Panis, Oxidative stress, redox signaling and cancer chemoresistance: putting together the pieces of the puzzle, *Curr. Med. Chem.* 21 (29) (2014) 3211–3226, <https://doi.org/10.2174/0929867321666140629161839>.
- [78] B. Griess, E. Tom, F. Domann, M. Teoh-Fitzgerald, Extracellular superoxide dismutase and its role in cancer, *Free Radic. Biol. Med.* 112 (2017) 464–479, <https://doi.org/10.1016/j.freeradbiomed.2017.08.013>.
- [79] L.L.D. Santos, A.T.F. Silva, I.C.C. Ferreira, A.V. Souza, A.B. Justino, D.W. Santos, L.R. Goulart, C.E. Paiva, F.S. Espíndola, Y.C.P. Maia, A lower serum antioxidant capacity as a distinctive feature for women with HER2+ breast cancer: a preliminary study, *Cancers* 14 (23) (2022) 5973, <https://doi.org/10.3390/cancers14235973>.
- [80] Z. Mohammadi Abgarmi, A. Sahebghadam Lotfi, S. Abroun, M. Soleimani, S. Mohammad Ganji, P. Baktash, A. Moradi, Breast cancer cell lines, HER2/Neu phenotype, and a higher propensity to reactive oxygen species production, *Archiv. Breast Cancer* 8 (2) (2021) 137–142, <https://doi.org/10.32768/abc.202182137-142>.
- [81] H. Jiang, J. Zuo, B. Li, R. Chen, K. Luo, X. Xiang, S. Lu, C. Huang, L. Liu, J. Tang, F. Gao, Drug-induced oxidative stress in cancer treatments: angel or devil? *Redox Biol.* 63 (2023) 102754 <https://doi.org/10.1016/j.redox.2023.102754>.
- [82] L.I. Gordon, M.A. Burke, A.T. Singh, S. Prachand, E.D. Lieberman, L. Sun, T. J. Naik, S.V. Prasad, H. Ardehali, Blockade of the erbB2 receptor induces cardiomyocyte death through mitochondrial and reactive oxygen species-dependent pathways, *J. Biol. Chem.* 284 (4) (2009) 2080–2087, <https://doi.org/10.1074/jbc.M804570200>.
- [83] R. Teppo, Y. Soini, P. Karihtala, Reactive oxygen species-mediated mechanisms of action of targeted cancer therapy, *Oxid. Med. Cell. Longev.* 2017 (2017) 1485283, <https://doi.org/10.1155/2017/1485283>.
- [84] H.J. Kang, Y.W. Yi, Y.B. Hong, H.J. Kim, Y.J. Jang, Y.S. Seong, I. Bae, HER2 confers drug resistance of human breast cancer cells through activation of NRF2 by direct interaction, *Sci. Rep.* 4 (2014) 7201, <https://doi.org/10.1038/srep07201>.
- [85] A. Sadeghirizi, R. Yazdanparast, S. Aghazadeh, Combating trastuzumab resistance by targeting thioredoxin-1/PTEN interaction, *Tumour Biol. : J. Int. Soc. Oncodevelopment. Biol. Med.* 37 (5) (2016) 6737–6747, <https://doi.org/10.1007/s13277-015-4424-9>.
- [86] F. Xing, Q. Hu, Y. Qin, J. Xu, B. Zhang, X. Yu, W. Wang, The relationship of redox with hallmarks of cancer: the importance of homeostasis and context, *Front. Oncol.* 12 (2022) 862743, <https://doi.org/10.3389/fonc.2022.862743>.
- [87] L. Milkovic, A. Cipak Gasparovic, M. Cindric, P.A. Mouthuy, N. Zarkovic, Short overview of ROS as cell function regulators and their implications in therapy concepts, *Cells* 8 (8) (2019) 793, <https://doi.org/10.3390/cells8080793>.
- [88] X. Chen, M. Song, B. Zhang, Y. Zhang, Reactive oxygen species regulate T cell immune response in the tumour microenvironment, *Oxid. Med. Cell. Longev.* (2016) 1580967, <https://doi.org/10.1155/2016/1580967>, 2016.
- [89] L.A. Sena, S. Li, A. Jairaman, M. Prakriya, T. Ezponda, D.A. Hildeman, C.R. Wang, P.T. Schumacker, J.D. Licht, H. Perlman, P.J. Bryce, N.S. Chandrel, Mitochondria are required for antigen-specific T cell activation through reactive oxygen species signaling, *Immunity* 38 (2) (2013) 225–236, <https://doi.org/10.1016/j.immuni.2012.10.020>.

- [90] K. Gölöw, D. Tümen, P. Heumann, S. Schmid, A. Kandulski, M. Müller, C. Kunst, Unraveling the role of reactive oxygen species in T lymphocyte signaling, *Int. J. Mol. Sci.* 25 (11) (2024) 6114, <https://doi.org/10.3390/ijms25116114>.
- [91] A. Allegra, G. Murdaca, G. Mirabile, S. Gangemi, Redox signaling modulates activity of immune checkpoint inhibitors in cancer patients, *Biomedicines* 11 (5) (2023) 1325, <https://doi.org/10.3390/biomedicines11051325>.
- [92] M. Oishi, S. Gandhi, L. Yan, Y. Tokumaru, R. Wu, A. Yamada, R. Matsuyama, I. Endo, K. Takabe, Abundance of reactive oxygen species (ROS) is associated with tumour aggressiveness, immune response, and worse survival in breast cancer, *Breast Cancer Res. Treat.* 194 (2) (2022) 231–241, <https://doi.org/10.1007/s10549-022-06633-0>.
- [93] Y. Bao, G. Oguz, W.C. Lee, P.L. Lee, K. Ghosh, J. Li, P. Wang, P.E. Lobie, S. Ehmsen, H.J. Ditzel, A. Wong, E.Y. Tan, S.C. Lee, Q. Yu, EZH2-mediated PP2A inactivation confers resistance to HER2-targeted breast cancer therapy, *Nat. Commun.* 11 (1) (2020) 5878, <https://doi.org/10.1038/s41467-020-19704-x>.
- [94] M. Wada, D. Canals, M. Adada, N. Coant, M.F. Salama, K.L. Helke, J.S. Arthur, K. R. Shroyer, K. Kitatani, L.M. Obeid, Y.A. Hannun, P38 delta MAPK promotes breast cancer progression and lung metastasis by enhancing cell proliferation and cell detachment, *Oncogene* 36 (47) (2017) 6649–6657, <https://doi.org/10.1038/onc.2017.274>.
- [95] M. Rojo de la Vega, E. Chapman, D.D. Zhang, NRF2 and the hallmarks of cancer, *Cancer Cell* 34 (1) (2018) 21–43, <https://doi.org/10.1016/j.ccr.2018.03.022>.
- [96] C. Gorriñi, P.S. Baniasadi, I.S. Harris, J. Silvester, S. Inoue, B. Snow, P.A. Joshi, A. Wakeham, S.D. Molyneux, B. Martin, P. Bouwman, D.W. Cescon, A.J. Elia, Z. Winterton-Perks, J. Cruickshank, D. Brenner, A. Tseng, M. Musgrave, H. K. Berman, R. Khokha, M.L. Gauthier, BRCA1 interacts with Nrf2 to regulate antioxidant signaling and cell survival, *J. Exp. Med.* 210 (8) (2013) 1529–1544, <https://doi.org/10.1084/jem.20121337>.
- [97] N.S. Clayton, E.P. Carter, A.E. Fearon, J.A. Heward, L. Rodríguez Fernández, L. Boughtetane, E.H. Wilkes, P.R. Cutillas, R.P. Grose, HDAC inhibition restores response to HER2-targeted therapy in breast cancer via PHLDA1 induction, *Int. J. Mol. Sci.* 24 (7) (2023) 6228, <https://doi.org/10.3390/ijms24076228>.
- [98] X. Li, J. Kuang, Y. Shen, M.M. Majer, C.C. Nelson, K. Parsawar, K.A. Heichman, S. K. Kuwada, The atypical histone macroH2A1.2 interacts with HER-2 protein in cancer cells, *J. Biol. Chem.* 287 (27) (2012) 23171–23183, <https://doi.org/10.1074/jbc.M112.379412>.
- [99] Z. Yang, J. Feng, J. Jing, Y. Huang, W.W. Ye, L. Lei, X.J. Wang, W.M. Cao, Resistance to anti-HER2 therapy associated with the TSC2 nonsynonymous variant c.4349 C > G (p.Pro1450Arg) is reversed by CDK4/6 inhibitor in HER2-positive breast cancer, *NPJ breast cancer* 9 (1) (2023) 36, <https://doi.org/10.1038/s41523-023-00542-1>.
- [100] A. Prat, V. Guarneri, T. Pascual, F. Brasó-Maristany, E. Sanfelix, L. Paré, F. Schettini, D. Martínez, P. Jares, G. Griguolo, M.V. Dieci, J. Cortés, A. Llombart-Cussac, B. Conte, M. Marín-Aguilera, N. Chic, J.A. Puig-Butillé, A. Martínez, P. Galván, Y.H. Tsai, C.M. Perou, Development and validation of the new HER2DX assay for predicting pathological response and survival outcome in early-stage HER2-positive breast cancer, *EBioMedicine* 75 (2022) 103801, <https://doi.org/10.1016/j.ebiom.2021.103801>.
- [101] X. Xu, S. Zhang, Y. Wang, Y. Zhu, J. Wang, J. Guo, HMOX1 pathway signature predicts clinical benefit from immunotherapy plus tyrosine kinase inhibitor therapy in advanced renal cell carcinoma, *Cancer Med.* 12 (9) (2023) 10512–10525, <https://doi.org/10.1002/cam4.5787>.
- [102] K.D. Sullivan, M.D. Galbraith, Z. Andryšík, J.M. Espinosa, Mechanisms of transcriptional regulation by p53, *Cell Death Differ.* 25 (1) (2018) 133–143, <https://doi.org/10.1038/cdd.2017.174>.
- [103] E. Sajjadi, K. Venetis, R. Piciotti, D. Gambini, C. Blundo, L. Runza, S. Ferrero, E. Guerini-Rocco, N. Fusco, Combined analysis of PTEN, HER2, and hormone receptors status: remodeling breast cancer risk profiling, *BMC Cancer* 21 (1) (2021) 1152, <https://doi.org/10.1186/s12885-021-08889-z>.
- [104] C.S. Abhinand, R. Raju, S.J. Soumya, P.S. Arya, P.R. Sudhakaran, VEGF-A/VEGFR2 signaling network in endothelial cells relevant to angiogenesis, *J. Cell Commun. Signal.* 10 (4) (2016) 347–354, <https://doi.org/10.1007/s12079-016-0352-8>.