



Article

Resistance to Combined Anthracycline–Taxane Chemotherapy Is Associated with Altered Metabolism and Inflammation in Breast Carcinomas

Otília Menyhárt ^{1,2,3}, János Tibor Fekete ^{1,2,3} and Balázs Györffy ^{1,2,3,*}

¹ Oncology Biomarker Research Group, Institute of Molecular Life Sciences, Hungarian Research Network, Magyar Tudósok Körútja 2, 1117 Budapest, Hungary; menyhart.otilia@med.semmelweis-univ.hu (O.M.); jfeketet@hotmail.com (J.T.F.)

² National Laboratory for Drug Research and Development, Magyar Tudósok Körútja 2, 1117 Budapest, Hungary

³ Department of Bioinformatics, Semmelweis University, 1094 Budapest, Hungary

* Correspondence: gyorffy.balazs@med.semmelweis-univ.hu

Abstract: Approximately 30% of early-stage breast cancer (BC) patients experience recurrence after systemic chemotherapy; thus, understanding therapy resistance is crucial in developing more successful treatments. Here, we investigated the mechanisms underlying resistance to combined anthracycline–taxane treatment by comparing gene expression patterns with subsequent therapeutic responses. We established a cohort of 634 anthracycline–taxane-treated patients with pathological complete response (PCR) and a separate cohort of 187 patients with relapse-free survival (RFS) data, each having transcriptome-level expression data of 10,017 unique genes. Patients were categorized as responders and non-responders based on their PCR and RFS status, and the expression for each gene was compared between the two groups using a Mann–Whitney U-test. Statistical significance was set at $p < 0.05$, with fold change (FC) > 1.44 . Altogether, 224 overexpressed genes were identified in the tumor samples derived from the patients without PCR; among these, the gene sets associated with xenobiotic metabolism (e.g., *CYP3A4*, *CYP2A6*) exhibited significant enrichment. The genes *ORAI3* and *BCAM* differentiated non-responders from responders with the highest AUC values ($AUC > 0.75$, $p < 0.0001$). We identified 51 upregulated genes in the tumor samples derived from the patients with relapse within 60 months, participating primarily in inflammation and innate immune responses (e.g., *LYN*, *LY96*, *ANXA1*). Furthermore, the amino acid transporter *SLC7A5*, distinguishing non-responders from responders, had significantly higher expression in tumors and metastases than in normal tissues (Kruskal–Wallis $p = 8.2 \times 10^{-20}$). The identified biomarkers underscore the significance of tumor metabolism and microenvironment in treatment resistance and can serve as a foundation for preclinical validation studies.

Keywords: breast cancer (BC); therapy resistance; anthracycline; taxane; inflammation; innate immune response



Citation: Menyhárt, O.; Fekete, J.T.; Györffy, B. Resistance to Combined Anthracycline–Taxane Chemotherapy Is Associated with Altered Metabolism and Inflammation in Breast Carcinomas. *Int. J. Mol. Sci.* **2024**, *25*, 1063. <https://doi.org/10.3390/ijms25021063>

Academic Editor: Ioannis Boutas

Received: 5 December 2023

Revised: 8 January 2024

Accepted: 11 January 2024

Published: 15 January 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Breast cancer (BC) is the most common malignancy among women and is the second most common cause of cancer death after lung cancer. In 2020, 2.3 million women were diagnosed worldwide with BC, and 685,000 patients succumbed to the disease [1]. With an incidence of 1 in 8, about 13% of women are diagnosed with BC in their lifetime in the United States, accounting for one-third of all new female malignancies [2]. Incidence rates, especially of the estrogen-positive subtype, are slightly increasing in Western countries (0.5% per year) [3]; however, mortality has steadily decreased since the 1980s, with a current 5-year survival rate of 90% when diagnosed at an early stage [4].

Treatment with neoadjuvant and adjuvant chemotherapy improves long-term survival and reduces the risk of BC recurrence. The choice of therapy depends on the patient's

general condition and co-morbidities, the presence of different risk factors, and the tumor type. For over a decade, combination therapies based on anthracyclines and taxanes have been the recommended treatment options for early-stage and high-risk BC [5]. According to the 2012 Early Breast Cancer Trialists' Collaborative Group (EBCTCG) meta-analysis, anthracycline-based regimens produced similar or better survival outcomes compared with cyclophosphamide, methotrexate, and fluorouracil (CMF) treatment, the historical standard of care; moreover, they found that adding taxane to anthracycline-based regimens further improved BC-specific and 10-year overall survival [5]. In a recent EBCTCG meta-analysis, taxanes combined with anthracyclines reduced recurrence rates compared with taxanes without anthracyclines. The highest benefits were observed when anthracyclines were added concomitantly to docetaxel plus cyclophosphamide, compared with docetaxel plus cyclophosphamide alone, leading to a 4.2 percent reduction in 10-year BC mortality (95% CI 0.4–8.1) [6]. Adding taxanes to anthracycline also reduced the 10-year risk of recurrence from 39% to 36% and the risk of BC-related mortality from 28% to 24 [6]. The effects of taxane plus anthracycline combinations were similar for estrogen receptor-positive and receptor-negative tumors, unaffected by age, lymph node status, tumor size, or grade; however, higher cumulative dose and higher-intensity treatments were more powerful [6].

Several mechanisms of action can explain the efficacy of anthracyclines: they intercalate the DNA and stabilize the topoisomerase II complex, stopping the replication process. Furthermore, anthracyclines promote cytotoxicity through free radicals while inducing histone detachment from chromatin, deregulating the epigenome and transcriptome. Thus, anthracycline treatment causes irreversible DNA damage, oxidative stress, and apoptotic cell death [7]. In contrast, taxanes bind with high affinity to microtubules and stabilize them, preventing mitotic spindle formation in the metaphase. The delayed activation of the mitotic checkpoint can trigger apoptosis [8].

Despite significant advances in the early detection of BC and a better understanding of underlying molecular mechanisms, 20–30% of BC patients are diagnosed in an advanced stage, and up to 30% of patients with early-stage breast carcinoma will relapse [9]. Although an initial clinical response and a reduction in the tumor size are frequent, the improvement is only temporary, as drug resistance can manifest within months of treatment. Consequently, innate or acquired drug resistance causes treatment failure in up to 90% of metastatic cancers [10–12]. Consequently, in many patients, neither a PCR nor a survival benefit can be derived from chemotherapy.

Over the past four decades, the mechanisms of chemoresistance have been extensively studied. It may involve changes in membrane transport and increased expulsion of drugs, mediated by members of the ATP-binding cassette (ABC) superfamily, particularly P-glycoprotein and multidrug-resistance-associated proteins (e.g., MDR1); this contributes toward both anthracycline and taxane resistance [13–16], along with other mechanisms related to drug metabolism [17]. Moreover, the effectiveness of anthracycline-induced cytotoxicity can be hindered by activated DNA repair mechanisms, modifications in the activity of topoisomerase II affecting the binding affinity between anthracyclines and TOP II, changes in apoptotic signaling pathways such as the tumor suppressor protein p53, alterations in cysteine and methionine metabolism, and the presence of genetic mutations [18–20].

Resistance to combined anthracycline–taxane treatment remains a significant obstacle in BC management. A better understanding of the mechanisms leading to resistance may improve BC treatment strategies and ultimately enhance patient survival. There is a crucial need for biomarkers that can predict therapeutic responses and aid patient classification. In this study, we aimed to identify genes that exhibit differential expression in patient samples obtained during surgery based on their subsequent response to anthracycline and taxane treatment. By comparing gene expression profiles of tumor samples, we identified genes that were upregulated in the tumors that were not responding to chemotherapy. Our findings indicate that a lack of PCR and disease recurrence is associated with the activity

of metabolic pathways and inflammatory and immune processes within the tumor and its microenvironment.

2. Results

2.1. Database for the Investigation of PCR

A database comprising 634 BC patients who received combined anthracycline–taxane treatment and had information on pathological response was constructed from the following Gene Expression Omnibus (GEO) and Array Express datasets: GSE18728, GSE25066, GSE41998, and E-TABM-43 (Figure 1A). Based on the St. Gallen Consensus classification, 30.6% of patients had triple-negative BC (TNBC), 33.1% were diagnosed with luminal A, 33.3% with luminal B, and 3% with HER2-positive BC subtype (Figure 1C). In contrast, based on gene expression, the majority of patients ($n = 421$, ~66%) were identified with estrogen-positive BC, and 28 patients (4.4%) had HER2-positive disease; of these, 9 patient samples (1.4%) were positive for both estrogen and HER2 receptors. A total of 294 cases were lymph-node-positive, while no lymph node involvement was present in 151 patients. Altogether, 28 patients were diagnosed with grade 1, 157 with grade 2, and 231 with grade 3 disease (Figure 1C). The mean age was 49.3 years (median 48, min 24, max 75 years) (Table 1).

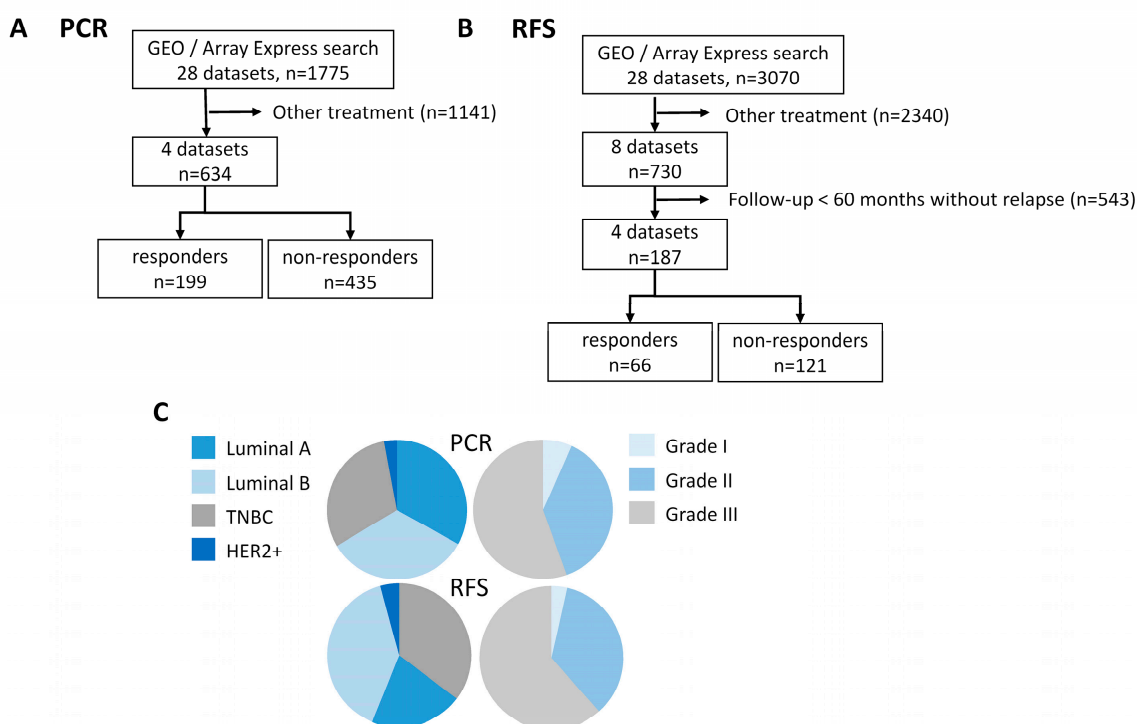


Figure 1. Database construction to investigate gene expression patterns related to (A) pathological complete response (PCR) and (B) recurrence-free survival (RFS). (C) Classification of the 634 patients in the PCR and 187 patients in RFS databases by St. Gallen Consensus subtype and tumor grade.

Most patients (96.7%) received neoadjuvant systemic treatment, while 3.3% were treated by adjuvant therapy. For most ($n = 448$) cases, the taxane–anthracyclin combination was the only known treatment. A total of 124 patients received neoadjuvant doxorubicin/cyclophosphamide followed by paclitaxel, and 61 patients (9.6%) were treated by adjuvant, or neoadjuvant combination of docetaxel, doxorubicin, capecitabine, and cyclophosphamide; a single patient received a neoadjuvant docetaxel–epirubicin combination (Table 1).

Of the 634 patients treated with systemic anthracycline–taxane therapy, only 31.4% developed a PCR.

Table 1. Patient characteristics in the PCR and RFS datasets.

		PCR	RFS
Number of patients		634	187
Responders		199	66
BC molecular subtypes (St. Gallen)			
	Luminal A	210	39
	Luminal B	211	74
	HER2+	19	8
	TNBC	194	66
Grade			
	I	28	6
	II	157	59
	III	231	104
	unavailable	218	18
Lymph node involvement			
	yes	294	138
	no	151	47
	unknown	189	2
Age (mean)		49.3 (min 24, max 75)	50.6 (min 24, max 75)
Treatment			
	taxane and anthracycline	448	187
	docetaxel, capecitabine (adriamycin and cyclophosphamide)	61	
	doxorubicin/cyclophosphamide followed by paclitaxel	124	
	docetaxel–epirubicin	1	

2.2. Genes Associated with the Lack of PCR

We compared gene expression from tumor samples between responders/non-responders and determined significantly upregulated genes in patients who did not develop a PCR ($p < 0.05$; FC > 1.44).

A total of 224 overexpressed genes were identified after p -value correction in tumor samples not developing PCR following an anthracycline–taxane treatment, out of which 135 genes had AUC values of 0.7 or higher (Supplementary Materials Table S1). The top three genes with the highest AUC values (*ORAI3*, *BCAM*, and *ATP6V0A1*) have been associated with cancer development. Nevertheless, the significance of these genes in restricted therapy response remains unknown, necessitating further functional investigations (Figure 2).

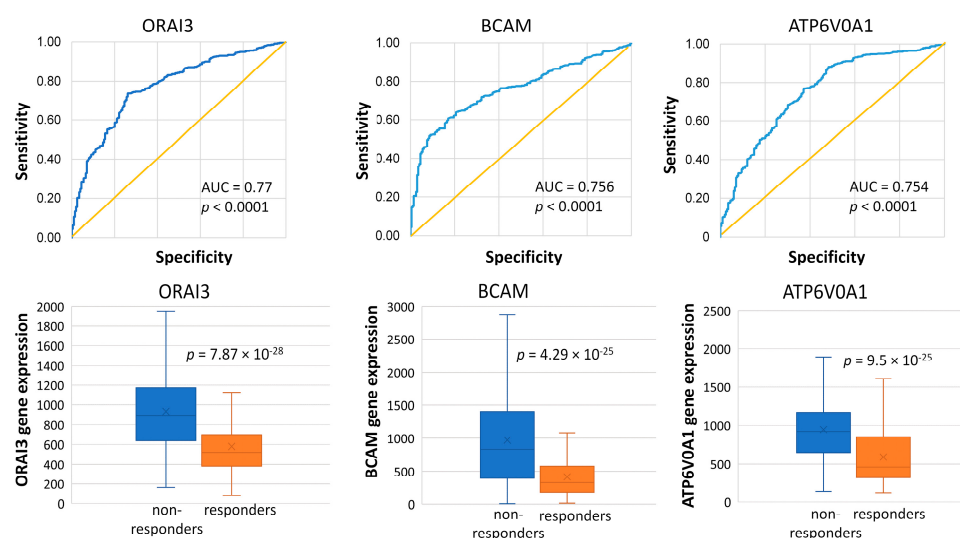


Figure 2. The high expression of *ORAI3*, *BCAM*, and *ATP6V0A1* differentiated non-responders from responders treated with anthracycline–taxane combination therapy in the PCR dataset (however, none

of these genes differentiated responders and non-responders in the RFS dataset, as illustrated in Supplementary Materials Table S2). The blue curve represents the discriminatory power of the model, and the yellow diagonal line serves as a baseline and represents random chance at 0.5.

2.3. Genes Associated with Tumor Progression

We assessed expression differences among the identified 224 genes in normal, tumor, and metastatic tissue samples using TNMplot [21] and identified genes exhibiting increasing gene expression. We found ten genes with significantly higher expression in tumors than normal samples and increasing expression in metastases (*HSPB1*, *G6PD*, *BSG*, *NAPA*, *KRT18*, *BLOC1S1*, *EMP3*, *CST3*, *RHOH*, and *NELL2*). Out of these genes, the expression of *G6PD*, *NAPA*, and *BSG* genes was the highest in tumors compared to normal tissues, with a further increase in metastatic samples and particularly high AUC values in the ROC analyses (Figure 3).

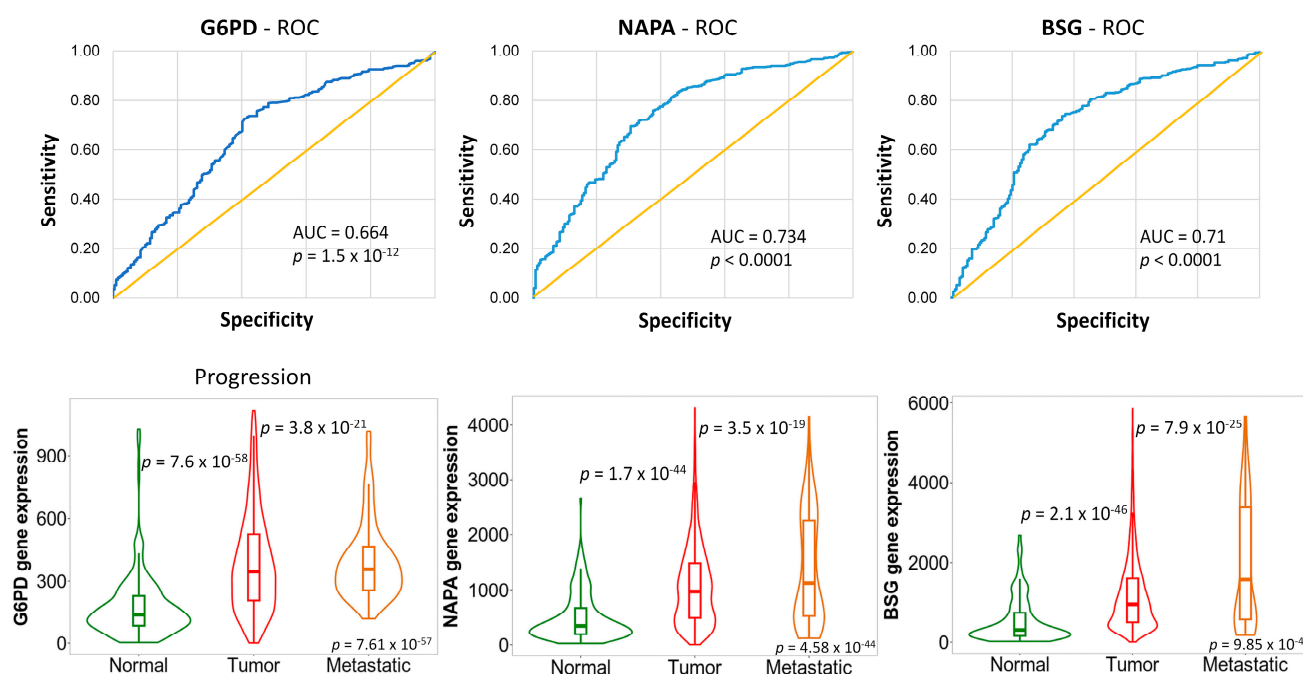


Figure 3. In the PCR database, high expression of *G6PD*, *NAPA*, and *BSG* genes significantly differentiated non-responders from responders. The blue curve represents the discriminatory power of the model, and the yellow diagonal line serves as a baseline and represents random chance at 0.5. *G6PD*, *NAPA*, and *BSG* genes were also associated with tumor progression, with elevated expression in tumors compared to normal samples, and a further increase in metastases. The p-values displayed in the bottom right-hand corner correspond to the Kruskal-Wallis test, assessing overall expression differences among normal, tumor, and metastatic samples. Pairwise comparisons between normal-tumor and tumor-metastatic samples were further scrutinized using the Dunn test.

2.4. Gene Ontology

Gene ontology analysis was performed using the top 135 upregulated genes with the strongest association to resistance ($AUC \geq 0.7$) using the DAVID bioinformatics tool. The most significantly enriched term was xenobiotic catabolism ($p = 3.28 \times 10^{-4}$), which included four differentially expressed genes: *CYP3A4* ($AUC = 0.704$, $p < 0.0001$), *CYP2A6* ($AUC = 0.7$, $p < 0.0001$) (Figure 4A), *CYP1A2* ($AUC = 0.704$, $p < 0.0001$), and *FMO4* ($AUC = 0.701$, $p < 0.0001$). The combined AUC score of these four genes was slightly greater than any of their individual values ($AUC = 0.719$, $p < 0.0001$), suggesting that the combined

expression of *CYP2A6*, *CYP1A2*, *CYP3A4*, and *FMO4* may be a valuable biomarker for patient stratification (Figure 4B).

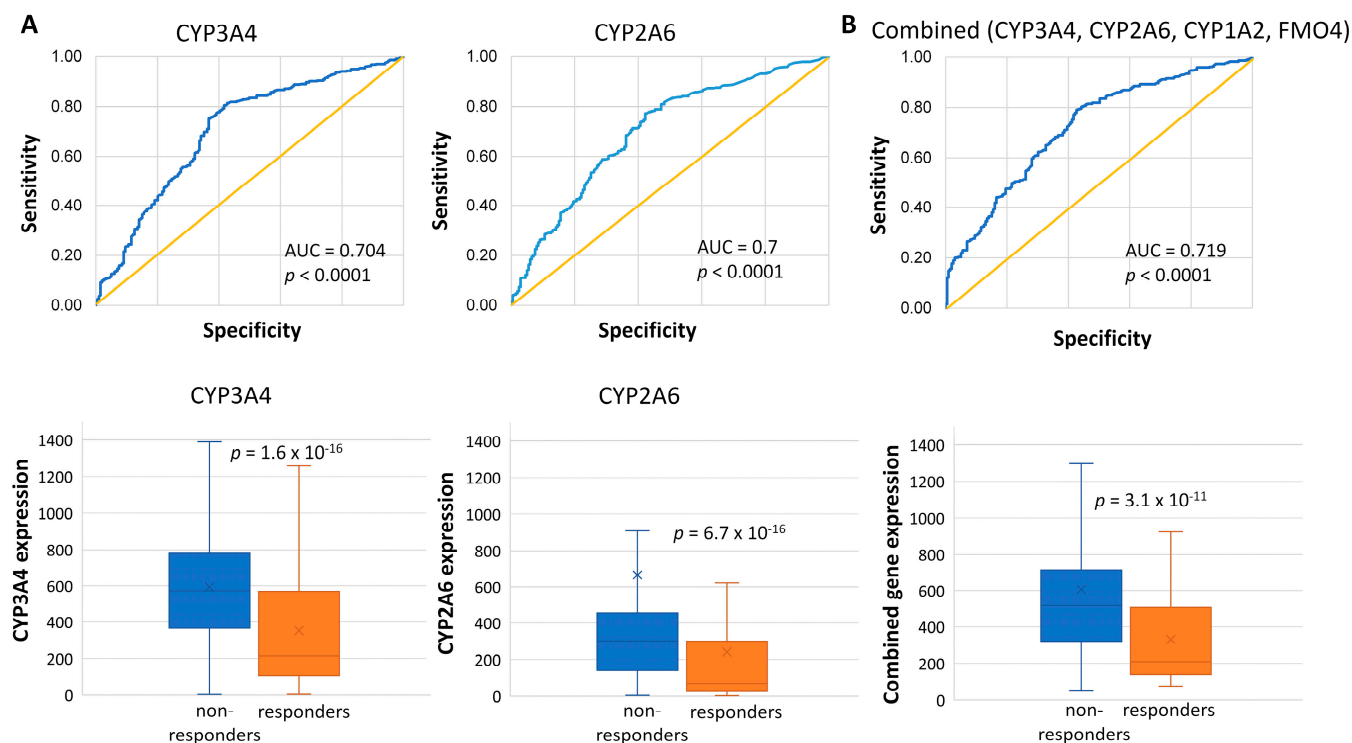


Figure 4. (A) According to DAVID gene ontology, *CYP* genes participating in xenobiotic catabolism, including *CYP3A4* and *CYP2A6*, were significantly overrepresented among the genes overexpressed in non-responders in the PCR database. (B) The combined AUC value of the *CYP* genes associated with xenobiotics catabolism was slightly higher than the AUC values of the individual genes.

2.5. Database for the Investigation of RFS

The data for a total of 187 BC patients treated exclusively with anthracyclines and paclitaxel, with available information on relapse-free survival (RFS), were extracted from the following datasets in GEO: GSE19615, GSE25066, GSE31519, and GSE65194 (Figure 1B). Over 90% of patients received neoadjuvant therapy. The mean RFS was 37.3 months (median 27.5, max: 89.3 months). If RFS > 60 months (5 years), patients were assigned to be responders. Of all patients, 64.7% (121) relapsed within 60 months from diagnosis.

Of the patients, 35.3% were identified to have TNBC, 20.9% were identified to have luminal A, 39.6% were identified to have luminal B, and 4.3% were identified to have HER2-positive disease (Figure 1C). In 138 cases, lymph node involvement was identified, while 47 patients were found to be node-negative. Grade 1 disease was identified in 6, grade 2 in 59, and grade 3 in 104 cases (Figure 1C). The mean age at diagnosis was 50.6 years (median 50.4, min: 24, max: 75 years) (Table 1).

2.6. Genes Associated with Disease Recurrence

Altogether, 51 genes had higher expression in tumor samples from patients who had relapsed within 60 months from diagnosis. We found a moderate association between the two endpoints (PCR and RFS) (Kappa = 0.241, $p = 0.000164$), with a 7.5-fold probability of developing RFS if PCR had already been reached (OR = 7.556, CI: 2.321–24.599).

We compared the list of candidate genes across the PCR and RFS groups. Interestingly, there were no overlaps between the lists of the upregulated 224 and 51 genes associated with either PCR or RFS, respectively. These genes perform entirely differently in the PCR and the RFS datasets; a comparison of their performance can be found in Supplementary Materials Table S2 (RFS vs. PCR) and Supplementary Materials Table S3 (PCR vs. RFS).

In the RFS dataset, based on AUC values, the *LYN*, *LY96*, and *HMOX1* genes separated non-responders from responders most successfully (*LYN* AUC = 0.753, $p = 3.5 \times 10^{-12}$; *LY96* AUC = 0.736, $p = 3.5 \times 10^{-12}$ (Figure 5), and *HMOX1* AUC = 0.738, $p = 3.5 \times 10^{-12}$).

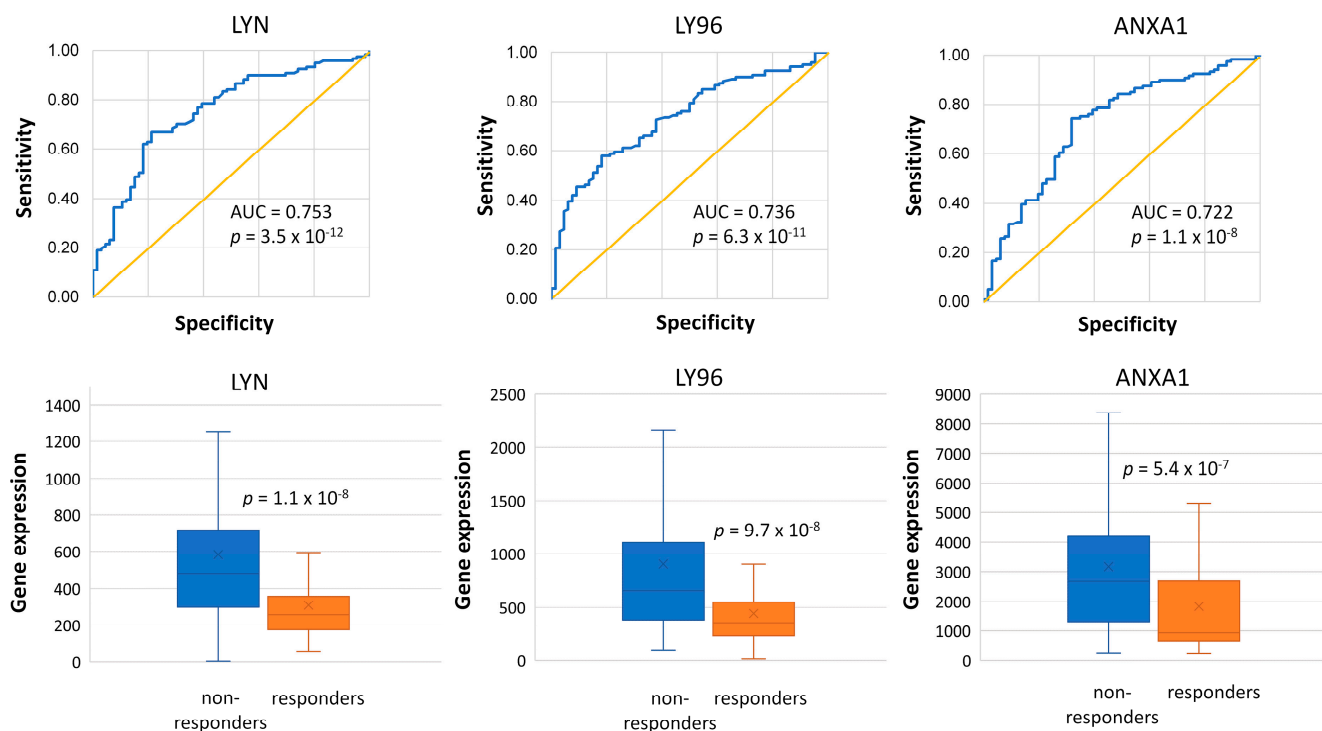


Figure 5. The high expression of *LYN*, *LY96*, and *ANXA1* genes were among the best that differentiated non-responders from responders treated by anthracycline-paclitaxel therapy in the RFS dataset.

2.7. Genes Associated with Tumor Progression

We evaluated expression differences among the identified 51 genes in normal, tumor, and metastatic tissue samples using TNMplot. Notably, the expression of the *SLC7A5* gene exhibited significant differences (Kruskal–Wallis test, $p = 8.2 \times 10^{-20}$): elevated expression was observed in tumor samples compared to normal tissues, with a further increase in metastases relative to tumors (Figure 6).

2.8. Inflammatory Processes Underlying Disease Recurrence

Gene ontology analysis by DAVID on the 51 genes revealed that inflammatory processes, innate immune response, and the Toll-like receptor 4 signaling pathway were the most significantly enriched terms (FDR < 13%) (Table 2). The *LYN*, *LY96*, and *ANXA1* genes assigned to these functions had exceptionally high AUC values separating responders and non-responders well in the ROC analysis (Figure 5).

Table 2. The genes significantly overexpressed in patients who relapsed after anthracycline-paclitaxel therapy showed enrichment of inflammatory processes and the innate immune response based on DAVID gene ontology.

GO Category	<i>p</i> -Value	Genes
Inflammatory response	4.19×10^{-4}	<i>CCR1</i> , <i>AIM2</i> , <i>ANXA1</i> , <i>LY96</i> , <i>CHI3L1</i> , <i>ADM</i> , <i>CD14</i>
Innate immune response	5.87×10^{-4}	<i>LYN</i> , <i>AIM2</i> , <i>ANXA1</i> , <i>MX2</i> , <i>SLA</i> , <i>LY96</i> , <i>DEFB1</i> , <i>CD14</i>
Toll-like receptor 4 signaling pathway	6.49×10^{-4}	<i>LYN</i> , <i>LY96</i> , <i>CD14</i>

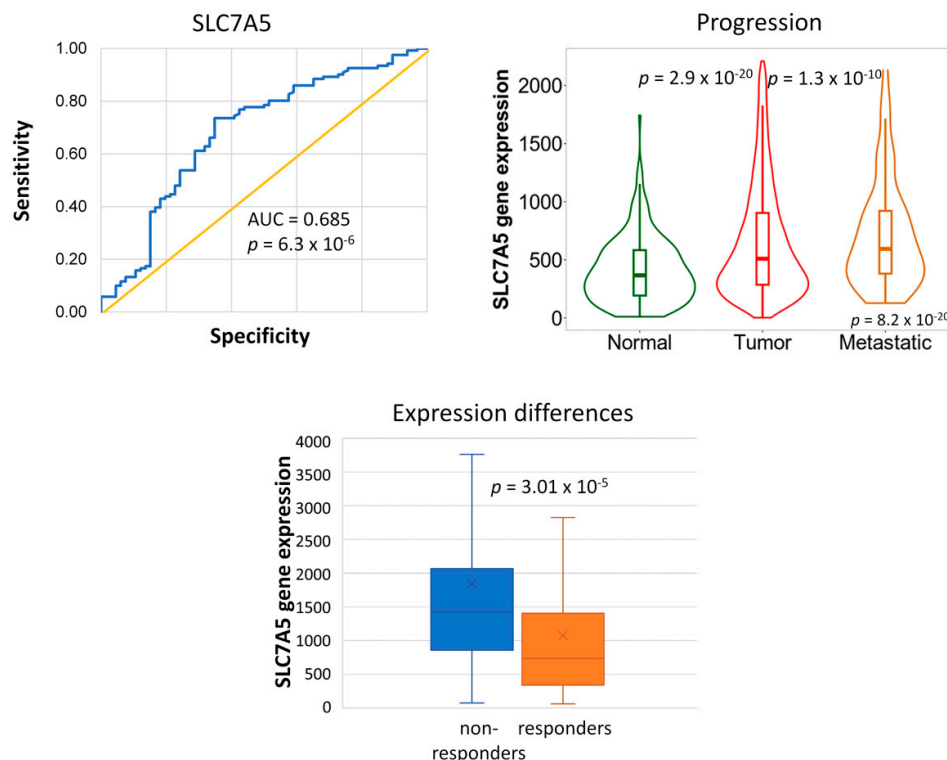


Figure 6. In the RFS dataset, 51 genes had higher expression in tumor samples from patients who had relapsed within 60 months from diagnosis. Among these genes, *SLC7A5* exhibited increased expression across normal samples, tumors, and metastases, as determined by TNMplot.com.

3. Discussion

We found that 68.6% out of the 634 patients treated with combined anthracyclin–taxane systemic therapy did not develop a PCR, and 64.7% out of 187 patients relapsed within five years after receiving the anthracyclin–paclitaxel combination, emphasizing the magnitude of therapy resistance in current BC treatment strategies. In clinical practice, PCR is a meaningful measure for assessing the efficacy of neoadjuvant treatment in patients with BC and strongly correlates with RFS and overall survival, particularly in the TNBCp and HER2-positive subtypes [22]. We found a moderate association between PCR and RFS; nevertheless, the genes associated with the two endpoints did not overlap. Notably, the treatment regimens administered in the two investigated patient populations were somewhat different: in the RFS group, patients were treated exclusively by anthracyclin–paclitaxel combinations, hindering direct comparisons with the PCR group.

Over 97% of the 634 patients received neoadjuvant chemotherapy in the PCR dataset and over 90% in the RFS dataset; thus, in most cases, tumor tissue was exposed to chemotherapy before surgery. Hence, the observed changes in gene expression primarily reflect the initial response to therapy.

Our results identified altered metabolic activity in tumors of non-responders. Extensive research focuses on the connection between metabolic pathways and the development of malignancies. Metabolic syndrome, characterized by conditions like dyslipidemia, hypertension, and type 2 diabetes, has been identified as a risk factor for BC [23]. Tumor cells exhibit increased glucose consumption and often depend on amino acids, particularly leucine, for protein synthesis and growth via the mTORC1 pathway [24].

We observed that high expression of the *SLC7A5* gene, encoding a plasma membrane amino acid transporter, was associated with tumor recurrence. *SLC7A5* is crucial in fulfilling cancer cells' elevated amino acid demand, particularly in TNBC, HER2-positive, and luminal B breast carcinomas [25] (the majority in our samples). Elevated *SLC7A5* expression correlates with larger tumor size, higher grade, distant metastases, and poor prognosis in estrogen-positive and HER2-positive BC subtypes [25]. High *SLC7A5* expression was

associated with resistance to chemotherapy in breast and other tumor types [26,27]. In lung carcinomas, increased *SLC7A5* expression contributes to an immunosuppressive tumor microenvironment and reduces the efficacy of immunotherapeutic treatments [28]. The inhibitors of *SLC7A5* currently being tested in clinical trials [26] could hold the potential to overcome drug resistance.

We also uncovered that a higher expression of the G6PD enzyme and genes encoding cytochrome P450 (CYP) enzymes involved in the breakdown of xenobiotics impacted the likelihood of achieving a PCR to anthracycline–taxane treatment. G6PD is involved in the pentose phosphate cycle, vital in generating ribose for DNA synthesis and maintaining redox balance through NADPH production. Increased G6PD expression has been linked to poor prognosis and resistance to chemotherapy in breast, colorectal, and lung carcinomas [29–32]. Inhibiting G6PD decreased cell viability, migration, and colonization ability while inducing autophagy through upregulating free radicals in BC cell cultures [32].

In our dataset, the high expression of genes participating in drug metabolisms, such as *CYP3A4*, *CYP2A6*, *CYP1A2*, and *FMO4*, negatively influenced the achievement of PCR. Cytochrome P450 (CYP)-dependent oxidases are heme-containing proteins involved in the degradation of compounds with an essential role in cellular signaling (including steroids, cholesterol, fatty acids, and eicosanoids), and detoxification of drugs [33]. Human *CYP3A4*, produced in high amounts in the liver and small intestine, is involved in the metabolism of most (~60%) of therapeutic drugs employed in the clinic [34]. CYP enzyme activity in tumors is highly relevant to the local metabolism of therapeutic agents, and many CYP genes have already been associated with the clinical efficacy of chemotherapy drugs [35]. CYP-enzymes are diverse, and their activity is context-dependent: in vitro, *CYP3A4* may accelerate tumor progression independently of oncogenes through arachidonic acid metabolism by activating the PI3K/AKT and STAT3 pathways [36] and stimulates angiogenesis through increased production of vascular endothelial growth factor (VEGF) [37]. *CYP3A4* protein can be detected in 20–55% of BC tissues [35], overexpressed mainly in stromal and glandular compartments [38], and in some cases, its high expression is linked to worse outcomes [39,40].

CYP3A4 and *CYP2A6* play a role in developing acquired resistance toward doxorubicin in luminal A-type BC cell lines [41], and *CYP3A4* also participates in taxane metabolism [35]. Increased CYP expression in tumors may limit the intracellular concentrations of docetaxel and paclitaxel, as their metabolites show no (or limited) cytotoxic activity [42,43]. CYP enzymes are activated by the human steroid xenobiotic receptor (SXR). Paclitaxel induces *CYP3A4* expression via the SXR receptor, thereby preventing its own uptake and increasing its excretion [35]. Accordingly, treatment with docetaxel in MCF7 cells caused elevated *CYP3A4* protein expression, which also occurred upon doxorubicin treatment [44]. Moreover, in clinical trials, patients with low *CYP3A4* expression had a significantly higher response rate to docetaxel [45]. In a separate study, patients with *CYP3A4*-negative tumors had responded better to docetaxel, associated with more prolonged progression-free survival compared to patients with *CYP3A4*-positive tumors (63.2% vs. 26.1%, $p < 0.01$) [46]. In our analysis, the combined expression of the four genes participating in xenobiotic catabolism (*CYP2A6*, *CYP1A2*, *CYP3A4*, and *FMO4*) differentiated non-responders from responders with a slightly higher AUC value than any of the separate genes, confirming that CYP enzyme expression in BC is a valuable tool for predicting tumor response to anthracycline–taxane protocols. Identifying previously established interactions between gene activity and therapy response reinforces the credibility of our approach.

Moreover, our data unveiled connections between genes whose roles in cancer initiation, progression, and therapy response remain relatively unexplored. Among these genes, namely *ORAI3*, *BCAM*, and *ATP6V0A1*, high expression levels provided the most effective differentiation between non-responders and responders in the PCR dataset, yielding AUC values ranging from 0.75 to 0.77. Notably, these genes serve entirely different functions: *ORAI3*, a calcium ion channel, has been associated with chemotherapy resistance due to its

upregulation, which hinders the expression of the p53 tumor suppressor gene and impedes apoptosis through a calcium-dependent mechanism. [47]. ORAI3, predominantly found in estrogen-positive breast tumors, promotes tumor cell division and cell cycle progression [48,49], underscoring the potential of ORAI3 as a biomarker of worse prognosis. The *BCAM* gene, also known as the basal cell adhesion molecule, encodes a protein member of the immunoglobulin superfamily and acts as a receptor for the extracellular matrix protein laminin. The function of the *BCAM* gene in BC is not well understood, but serum *BCAM* levels are elevated in BC patients [50]. The *ATP6V0A1* gene encodes a subunit of the vacuolar ATPase (V-ATPase) proton pump, and its role in BC requires further research. The significance of these genes in restricted therapy response remains unclear, necessitating further exploration through additional functional investigations. A distinctive strength in our study lies in proposing previously unexplored biomarker candidates, providing avenues for future validations.

In relapsed patients, we also identified an upregulated expression of genes linked to inflammatory processes and the innate immune response. In BC, inflammation is an important prognostic marker, as inflammatory cells produce factors that stimulate vascular and extracellular matrix remodeling [51]. Moreover, anthracyclines impact the immune-environment as their anticancer activity is mediated by the adaptive antitumor immune responses [52]. Previous studies uncovered markedly different immune infiltration patterns between anthracyclin-sensitive and resistant BC populations [19]. In our dataset, genes such as *LYN*, *LY96*, *ANXA1*, and *CCR1*, linked to inflammatory processes and the innate immune response, effectively distinguished patients who experienced recurrence from responders, as indicated by their AUC values above 0.72 in the ROC analysis.

LYN, a member of the Src receptor kinase family, has been extensively studied in BCR-ABL+ leukemia, where its persistent activation is linked to imatinib resistance [53]. In TNBC, elevated expression of *LYN* has been consistently linked to epithelial-mesenchymal transition, influencing cell invasion and metastatic potential [54]. Its significance extends beyond TNBC, as a high *LYNA*/*LYNB* isoform ratio is associated with poor prognosis in all breast carcinomas [55]. *LY96*, also known as Myeloid Differentiation Factor 2 (MD-2), is a glycoprotein involved in the initial steps of immune defense against mycobacteria. It acts as a co-receptor of Toll-like receptor 4 (TLR4), facilitating its activation through binding to its extracellular domain, initiating the innate immune response to lipopolysaccharides (LPSs) found on the outer membrane of Gram-negative bacteria [56]. *ANXA1* gene, overexpressed in non-responders in our dataset, is also involved in TLR4 regulation and the production of IFN- β induced by TLR4 [57].

One of our study's main limitations is that we could not conduct subtype-specific investigations due to the limited sample size. Moreover, it should be noted that our dataset does not reflect the prevalence of breast tumor subtypes: the TNBC subtype accounts for 10–15% of all breast tumors, while the proportion of HER2-positive breast carcinomas is around 15–20%. In contrast, over 30% of patients in our database belonged to the TNBC subtype, while the proportion of HER2-positive patients was only 4%.

Based on the literature data, patients diagnosed with the TNBC subtype are expected to have the best response to systemic chemotherapy (e.g., anthracycline–taxane) [58]. In our dataset, of the 187 patients, 33% with the TNBC subtype responded to treatment in the long term. This value is close to the response rate described in the literature, which, depending on treatment, ranges from 30 and 50% for PCR in this subtype [58,59]. Therefore, the non-representative subtype distribution from the available data provides insight mainly into resistance mechanisms in TNBC and estrogen-positive, especially luminal B tumors. Nevertheless, according to previous findings in the literature, many identified markers upregulated in our non-responder datasets (e.g., *LYN*) are relevant for a broad spectrum of breast tumors.

Our findings align with prior research emphasizing the significance of various biological processes, including metabolism and immune responses [60]. In particular, our study complements previous investigations, predominantly conducted in vitro, which

explored alterations in gene expression within cell cultures following doxorubicin or taxane monotherapy [60,61]. Mouse studies have implicated the modified activity of the MAPK/ERK pathway in developing resistance to doxorubicin [62]. Notably, our current study, utilizing gene expression patterns from treated tumors, provides a more nuanced understanding of the interactions between tumor cells and their immediate microenvironment, potentially accounting for identifying additional biomarkers distinct from those reported in previous in vitro studies.

4. Materials and Methods

4.1. Database Construction

To obtain datasets suitable for analyzing the therapeutic response, we searched the GEO (<http://www.pubmed.com/geo>, accessed on 20 March 2023) and Array Express (<https://www.ebi.ac.uk/biostudies/arrayexpress>, accessed on 22 March 2023) databases using specific keywords, including “breast”, “cancer”, “survival”, “GPL96”, “GPL570”, and “GPL571”. We focused on three Affymetrix gene array platforms (GPL96, GPL570, and GPL571) that employ identical hybridization probes for measuring gene expression. Our criteria for selecting publications included the availability of raw microarray gene expression data and clinical information regarding treatment and response. GEO datasets containing fewer than 20 samples were omitted. Some datasets initially included more than 20 specimens, but only a limited number of patients were relevant to our study. Repeatedly published arrays were identified by searching for identical expression values; only the initial one was included in the final database.

The raw CEL files were downloaded and subjected to MAS5 normalization using the Affy Bioconductor library within the R statistical environment (<http://www.r-project.org>, accessed on 30 March 2023) [63]. The MAS5 algorithm is designed explicitly for microarray data. It employs background correction, probe-level summarization, and global scaling to provide reliable and comparable gene expression values while addressing background noise and systematic variations within microarray datasets. We further performed scaling normalization to minimize variation between runs, setting the average expression on each chip to a standardized value of 1000. Affymetrix gene expression microarrays often detect a single gene using multiple probe sets; consequently, obtaining an unambiguous and reliable expression estimate for a specific gene can be challenging. Therefore, we employed a JetSet filter to fit a single probe to each gene [64].

4.2. Clinical Data

Individuals' clinical data were collected and validated by two researchers (B.G. and J.T.F.) to ensure the accuracy and reliability of the clinical information for each sample. The clinical data examined the association between initial gene expression in tumor samples and subsequent progression among patients treated with anthracycline–taxane combination therapy. Two endpoints were defined: the absence/presence of pathological complete response (PCR) and the length of relapse-free survival (RFS). For RFS, the cutoff value was set at 60 months, and patients with RFS > 60 months were defined as responders. Patient samples censored before 60 months were excluded from the analysis.

4.3. Selection of Genes Associated with Treatment Response

A database with 634 patient samples was constructed to investigate genes associated with PCR, and another database with 187 samples was created to study RFS (Figure 1A,B). Gene expression information was available from these samples for 22,277 probes, representing 10,017 unique genes after applying the JetSet filter. Expression for each gene was compared between responders and non-responders for the two endpoints (PCR, RFS) by using the Mann–Whitney U-test and receiver operator characteristics (ROC). Statistical significance was accepted at $p < 0.05$ if the fold change (FC) was >1.44. In addition, we utilized the Hochberg method for p -value correction [65]. Only genes with a mean expression above 500 (corresponding to 50% of the all-genes mean) among non-responders were considered

to highlight genes with potential relevance for future therapeutic investigations. Analysis was performed in the R statistical environment using components of the Bioconductor software package (BiocManager version: 3.18) [66].

4.4. Gene Ontology

We conducted gene ontology analysis on the upregulated genes observed in non-responders using the Database for Annotation, Visualization, and Integrated Discovery (DAVID) gene ontology database version 2021 [67].

4.5. Genes Associated with Tumor Progression

We evaluated the extent of expression differences in the identified candidate genes that distinguish responders from non-responders across normal, tumor, and metastatic tissue samples using TNMplot (www.tnmplot.com, accessed on 1 July 2023) [21] and searched for genes with increasing gene expression. We selected genes with mean expression > 500 in tumors and FC values > 1.44 between normal and tumor samples, with a further increase in metastases. For *p*-value correction, we used a 5% false discovery rate (FDR).

5. Conclusions

In conclusion, our study revealed that a substantial proportion of patients (68.6%) treated with combined anthracycline–taxane systemic therapy did not achieve a PCR, and a significant portion (64.7%) experienced relapse within five years, underscoring the prevalent resistance to current BC treatment strategies. Investigating gene upregulation in non-responders revealed a critical role of the modified immune environment, inflammation, and metabolism, validating some previously identified biomarkers associated with resistance to anthracycline–taxane treatment. However, the significance of many of these genes in restricted therapy response remains unclear, warranting further in-depth functional investigations. A distinctive strength of our study lies in proposing novel biomarker candidates, opening avenues for future validations to enhance patient stratification and establish a foundation for continued research in this field.

Supplementary Materials: The supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijms25021063/s1>.

Author Contributions: Conceptualization, O.M., B.G. and J.T.F.; methodology, B.G. and J.T.F.; data curation, J.T.F.; validation, B.G. and J.T.F.; formal analysis, O.M. and J.T.F.; writing—original draft preparation, O.M.; writing—review and editing, O.M., B.G. and J.T.F.; visualization, O.M. and J.T.F.; supervision, B.G.; funding acquisition, B.G. and O.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the National Research, Development and Innovation Office of Hungary (grant name: PharmaLab, RRF-2.3.1-21-2022-00015). O.M. was supported by the Janos Bolyai Scholarship of the Hungarian Academy of Sciences.

Institutional Review Board Statement: Not applicable, the analysis was conducted on publicly available data.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Arnold, M.; Morgan, E.; Rumgay, H.; Mafra, A.; Singh, D.; Laversanne, M.; Vignat, J.; Gralow, J.R.; Cardoso, F.; Siesling, S.; et al. Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast* **2022**, *66*, 15–23. [CrossRef] [PubMed]
2. Kocarnik, J.M.; Compton, K.; Dean, F.E.; Fu, W.; Gaw, B.L.; Harvey, J.D.; Henrikson, H.J.; Lu, D.; Pennini, A.; Xu, R.; et al. Cancer Incidence, Mortality, Years of Life Lost, Years Lived With Disability, and Disability-Adjusted Life Years for 29 Cancer Groups From 2010 to 2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *JAMA Oncol.* **2022**, *8*, 420–444.

3. Mesa-Eguiaagaray, I.; Wild, S.H.; Rosenberg, P.S.; Bird, S.M.; Brewster, D.H.; Hall, P.S.; Cameron, D.A.; Morrison, D.; Figueroa, J.D. Distinct temporal trends in breast cancer incidence from 1997 to 2016 by molecular subtypes: A population-based study of Scottish cancer registry data. *Br. J. Cancer* **2020**, *123*, 852–859. [[CrossRef](#)] [[PubMed](#)]
4. Giaquinto, A.N.; Sung, H.; Miller, K.D.; Kramer, J.L.; Newman, L.A.; Minihan, A.; Jemal, A.; Siegel, R.L. Breast Cancer Statistics, 2022. *CA A Cancer J. Clin.* **2022**, *72*, 524–541. [[CrossRef](#)]
5. Peto, R.; Davies, C.; Godwin, J.; Gray, R.; Pan, H.C.; Clarke, M. Comparisons between different polychemotherapy regimens for early breast cancer: Meta-analyses of long-term outcome among 100,000 women in 123 randomised trials. *Lancet* **2012**, *379*, 432–444. [[PubMed](#)]
6. Early Breast Cancer Trialists' Collaborative Group. Anthracycline-containing and taxane-containing chemotherapy for early-stage operable breast cancer: A patient-level meta-analysis of 100,000 women from 86 randomised trials. *Lancet* **2023**, *401*, 1277–1292. [[CrossRef](#)] [[PubMed](#)]
7. Beretta, G.L.; Zunino, F. Molecular mechanisms of anthracycline activity. *Top. Curr. Chem.* **2008**, *283*, 1–19.
8. Abal, M.; Andreu, J.M.; Barasoain, I. Taxanes: Microtubule and centrosome targets, and cell cycle dependent mechanisms of action. *Curr. Cancer Drug Targets* **2003**, *3*, 193–203. [[CrossRef](#)]
9. Siegel, R.L.; Miller, K.D.; Fuchs, H.E.; Jemal, A. Cancer statistics, 2022. *CA A Cancer J. Clin.* **2022**, *72*, 7–33. [[CrossRef](#)]
10. Menyhárt, O.; Santarpia, L.; Györfy, B. A Comprehensive Outline of Trastuzumab Resistance Biomarkers in HER2 Overexpressing Breast Cancer. *Curr. Cancer Drug Targets* **2015**, *15*, 665–683. [[CrossRef](#)]
11. Garrett, J.T.; Arteaga, C.L. Resistance to HER2-directed antibodies and tyrosine kinase inhibitors: Mechanisms and clinical implications. *Cancer Biol. Ther.* **2011**, *11*, 793–800. [[CrossRef](#)] [[PubMed](#)]
12. Longley, D.B.; Johnston, P.G. Molecular mechanisms of drug resistance. *J. Pathol.* **2005**, *205*, 275–292. [[CrossRef](#)] [[PubMed](#)]
13. Burger, H.; Foekens, J.A.; Look, M.P.; Meijer-van Gelder, M.E.; Klijn, J.G.; Wiemer, E.A.; Stoter, G.; Nooter, K. RNA expression of breast cancer resistance protein, lung resistance-related protein, multidrug resistance-associated proteins 1 and 2, and multidrug resistance gene 1 in breast cancer: Correlation with chemotherapeutic response. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **2003**, *9*, 827–836.
14. Mechetner, E.; Kyshtobayeva, A.; Zonis, S.; Kim, H.; Stroup, R.; Garcia, R.; Parker, R.J.; Fruehauf, J.P. Levels of multidrug resistance (MDR1) P-glycoprotein expression by human breast cancer correlate with in vitro resistance to taxol and doxorubicin. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **1998**, *4*, 389–398.
15. Pasquier, J.; Magal, P.; Boulangé-Lecomte, C.; Webb, G.; Le Foll, F. Consequences of cell-to-cell P-glycoprotein transfer on acquired multidrug resistance in breast cancer: A cell population dynamics model. *Biol. Direct* **2011**, *6*, 5. [[CrossRef](#)]
16. Harbottle, A.; Daly, A.K.; Atherton, K.; Campbell, F.C. Role of glutathione S-transferase P1, P-glycoprotein and multidrug resistance-associated protein 1 in acquired doxorubicin resistance. *Int. J. Cancer* **2001**, *92*, 777–783. [[CrossRef](#)] [[PubMed](#)]
17. Guo, B.; Tam, A.; Santi, S.A.; Parissenti, A.M. Role of autophagy and lysosomal drug sequestration in acquired resistance to doxorubicin in MCF-7 cells. *BMC Cancer* **2016**, *16*, 762. [[CrossRef](#)]
18. Aas, T.; Børresen, A.L.; Geisler, S.; Smith-Sørensen, B.; Johnsen, H.; Varhaug, J.E.; Akslen, L.A.; Lønning, P.E. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat. Med.* **1996**, *2*, 811–814. [[CrossRef](#)]
19. Liu, Z.; Gao, J.; Gu, R.; Shi, Y.; Hu, H.; Liu, J.; Huang, J.; Zhong, C.; Zhou, W.; Yang, Y.; et al. Comprehensive Analysis of Transcriptomics and Genetic Alterations Identifies Potential Mechanisms Underlying Anthracycline Therapy Resistance in Breast Cancer. *Biomolecules* **2022**, *12*, 1834. [[CrossRef](#)]
20. Knappskog, S.; Leirvaag, B.; Gansmo, L.B.; Romundstad, P.; Hveem, K.; Vatten, L.; Lønning, P.E. Prevalence of the CHEK2 R95* germline mutation. *Hered. Cancer Clin. Pract.* **2016**, *14*, 19. [[CrossRef](#)]
21. Bartha, Á.; Györfy, B. TNMplot.com: A Web Tool for the Comparison of Gene Expression in Normal, Tumor and Metastatic Tissues. *Int. J. Mol. Sci.* **2021**, *22*, 2622. [[CrossRef](#)] [[PubMed](#)]
22. Spring, L.M.; Fell, G.; Arfe, A.; Sharma, C.; Greenup, R.A.; Reynolds, K.L.; Smith, B.L.; Alexander, B.M.; Moy, B.; Isakoff, S.J.; et al. Pathological complete Response after Neoadjuvant Chemotherapy and Impact on Breast Cancer Recurrence and Survival: A Comprehensive Meta-analysis. *Clin. Cancer Res. Off. J. Am. Assoc. Cancer Res.* **2020**, *26*, 2838–2848. [[CrossRef](#)] [[PubMed](#)]
23. Dibaba, D.T.; Ogunsina, K.; Braithwaite, D.; Akinyemiju, T. Metabolic syndrome and risk of breast cancer mortality by menopause, obesity, and subtype. *Breast Cancer Res. Treat.* **2019**, *174*, 209–218. [[CrossRef](#)]
24. Bond, P. Regulation of mTORC1 by growth factors, energy status, amino acids and mechanical stimuli at a glance. *J. Int. Soc. Sports Nutr.* **2016**, *13*, 8. [[CrossRef](#)] [[PubMed](#)]
25. El Ansari, R.; Craze, M.L.; Miligy, I.; Diez-Rodriguez, M.; Nolan, C.C.; Ellis, I.O.; Rakha, E.A.; Green, A.R. The amino acid transporter SLC7A5 confers a poor prognosis in the highly proliferative breast cancer subtypes and is a key therapeutic target in luminal B tumours. *Breast Cancer Res.* **2018**, *20*, 21. [[CrossRef](#)] [[PubMed](#)]
26. Saito, Y.; Soga, T. Amino acid transporters as emerging therapeutic targets in cancer. *Cancer Sci.* **2021**, *112*, 2958–2965. [[CrossRef](#)] [[PubMed](#)]
27. Sato, M.; Harada-Shoji, N.; Toyohara, T.; Soga, T.; Itoh, M.; Miyashita, M.; Tada, H.; Amari, M.; Anzai, N.; Furumoto, S.; et al. L-type amino acid transporter 1 is associated with chemoresistance in breast cancer via the promotion of amino acid metabolism. *Sci. Rep.* **2021**, *11*, 589. [[CrossRef](#)]
28. Liu, Y.; Ma, G.; Liu, J.; Zheng, H.; Huang, G.; Song, Q.; Pang, Z.; Du, J. SLC7A5 is a lung adenocarcinoma-specific prognostic biomarker and participates in forming immunosuppressive tumor microenvironment. *Heliyon* **2022**, *8*, e10866. [[CrossRef](#)]

29. Ju, H.-Q.; Lu, Y.-X.; Wu, Q.-N.; Liu, J.; Zeng, Z.-L.; Mo, H.-Y.; Chen, Y.; Tian, T.; Wang, Y.; Kang, T.-B.; et al. Disrupting G6PD-mediated Redox homeostasis enhances chemosensitivity in colorectal cancer. *Oncogene* **2017**, *36*, 6282–6292. [\[CrossRef\]](#)
30. Pu, H.; Zhang, Q.; Zhao, C.; Shi, L.; Wang, Y.; Wang, J.; Zhang, M. Overexpression of G6PD is associated with high risks of recurrent metastasis and poor progression-free survival in primary breast carcinoma. *World J. Surg. Oncol.* **2015**, *13*, 323. [\[CrossRef\]](#)
31. Nagashio, R.; Oikawa, S.; Yanagita, K.; Hagiuda, D.; Kuchitsu, Y.; Igawa, S.; Naoki, K.; Satoh, Y.; Ichinoe, M.; Murakumo, Y.; et al. Prognostic significance of G6PD expression and localization in lung adenocarcinoma. *Biochim. Biophys. Acta Proteins Proteom.* **2019**, *1867*, 38–46. [\[CrossRef\]](#) [\[PubMed\]](#)
32. Li, Y.; Zheng, F.; Zhang, Y.; Lin, Z.; Yang, J.; Han, X.; Feng, Y.; Pei, X.; Li, F.; Liu, Q.; et al. glucose-6-phosphate dehydrogenase by 6-AN induces ROS-mediated autophagic cell death in breast cancer. *FEBS J.* **2023**, *290*, 763–779. [\[CrossRef\]](#)
33. Wrighton, S.A.; VandenBranden, M.; Ring, B.J. The human drug metabolizing cytochromes P450. *J. Pharmacokinet. Biopharm.* **1996**, *24*, 461–473. [\[CrossRef\]](#) [\[PubMed\]](#)
34. Guengerich, F.P. Cytochrome P-450 3A4: Regulation and role in drug metabolism. *Annu. Rev. Pharmacol. Toxicol.* **1999**, *39*, 1–17. [\[CrossRef\]](#) [\[PubMed\]](#)
35. van Eijk, M.; Boosman, R.J.; Schinkel, A.H.; Huitema, A.D.R.; Beijnen, J.H. Cytochrome P450 3A4, 3A5, and 2C8 expression in breast, prostate, lung, endometrial, and ovarian tumors: Relevance for resistance to taxanes. *Cancer Chemother. Pharmacol.* **2019**, *84*, 487–499. [\[CrossRef\]](#)
36. Mitra, R.; Guo, Z.; Milani, M.; Mesaros, C.; Rodriguez, M.; Nguyen, J.; Luo, X.; Clarke, D.; Lamba, J.; Schuetz, E.; et al. CYP3A4 Mediates Growth of Estrogen Receptor-positive Breast Cancer Cells in Part by Inducing Nuclear Translocation of Phospho-Stat3 through Biosynthesis of (±)-14,15-Epoxyeicosatrienoic Acid (EET)*. *J. Biol. Chem.* **2011**, *286*, 17543–17559. [\[CrossRef\]](#) [\[PubMed\]](#)
37. Klyushova, L.S.; Perepechaeva, M.L.; Grishanova, A.Y. The Role of CYP3A in Health and Disease. *Biomedicines* **2022**, *10*, 2686. [\[CrossRef\]](#)
38. Floriano-Sanchez, E.; Rodriguez, N.C.; Bandala, C.; Coballase-Urrutia, E.; Lopez-Cruz, J. CYP3A4 expression in breast cancer and its association with risk factors in Mexican women. *Asian Pac. J. Cancer Prev. APJCP* **2014**, *15*, 3805–3809. [\[CrossRef\]](#)
39. Murray, G.I.; Patimalla, S.; Stewart, K.N.; Miller, I.D.; Heys, S.D. Profiling the expression of cytochrome P450 in breast cancer. *Histopathology* **2010**, *57*, 202–211. [\[CrossRef\]](#)
40. Haas, S.; Pierl, C.; Harth, V.; Pesch, B.; Rabstein, S.; Brüning, T.; Ko, Y.; Hamann, U.; Justenhoven, C.; Brauch, H.; et al. Expression of xenobiotic and steroid hormone metabolizing enzymes in human breast carcinomas. *Int. J. Cancer* **2006**, *119*, 1785–1791. [\[CrossRef\]](#)
41. Barata, I.S.; Gomes, B.C.; Rodrigues, A.S.; Rueff, J.; Kranendonk, M.; Esteves, F. The Complex Dynamic of Phase I Drug Metabolism in the Early Stages of Doxorubicin Resistance in Breast Cancer Cells. *Genes* **2022**, *13*, 1977. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Sparreboom, A.; Huizing, M.T.; Boesen, J.J.B.; Nooijen, W.J.; van Tellingen, O.; Beijnen, J.H. Isolation, purification, and biological activity of mono- and dihydroxylated paclitaxel metabolites from human feces. *Cancer Chemother. Pharmacol.* **1995**, *36*, 299–304. [\[CrossRef\]](#) [\[PubMed\]](#)
43. Sparreboom, A.; Van Tellingen, O.; Scherrenburg, E.J.; Boesen, J.J.; Huizing, M.T.; Nooijen, W.J.; Versluis, C.; Beijnen, J.H. Isolation, purification and biological activity of major docetaxel metabolites from human feces. *Drug Metab. Dispos. Biol. Fate Chem.* **1996**, *24*, 655–658. [\[PubMed\]](#)
44. Li, W.-J.; Zhong, S.-L.; Wu, Y.-J.; Xu, W.-D.; Xu, J.-J.; Tang, J.-H.; Zhao, J.-H. Systematic expression analysis of genes related to multidrug-resistance in isogenic docetaxel- and adriamycin-resistant breast cancer cell lines. *Mol. Biol. Rep.* **2013**, *40*, 6143–6150. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Miyoshi, Y.; Ando, A.; Takamura, Y.; Taguchi, T.; Tamaki, Y.; Noguchi, S. Prediction of response to docetaxel by CYP3A4 mRNA expression in breast cancer tissues. *Int. J. Cancer* **2002**, *97*, 129–132. [\[CrossRef\]](#) [\[PubMed\]](#)
46. Sakurai, K.; Enomoto, K.; Matsuo, S.; Amano, S.; Shiono, M. CYP3A4 expression to predict treatment response to docetaxel for metastasis and recurrence of primary breast cancer. *Surg. Today* **2011**, *41*, 674–679. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Faouzi, M.; Hague, F.; Potier, M.; Ahidouch, A.; Sevestre, H.; Ouadid-Ahidouch, H. Down-regulation of Orai3 arrests cell-cycle progression and induces apoptosis in breast cancer cells but not in normal breast epithelial cells. *J. Cell. Physiol.* **2011**, *226*, 542–551. [\[CrossRef\]](#)
48. Faouzi, M.; Kischel, P.; Hague, F.; Ahidouch, A.; Benzerdjeb, N.; Sevestre, H.; Penner, R.; Ouadid-Ahidouch, H. ORAI3 silencing alters cell proliferation and cell cycle progression via c-myc pathway in breast cancer cells. *Biochim. Biophys. Acta* **2013**, *1833*, 752–760. [\[CrossRef\]](#)
49. Hasna, J.; Hague, F.; Rodat-Despoix, L.; Geerts, D.; Leroy, C.; Tulasne, D.; Ouadid-Ahidouch, H.; Kischel, P. Orai3 calcium channel and resistance to chemotherapy in breast cancer cells: The p53 connection. *Cell Death Differ.* **2018**, *25*, 693–707. [\[CrossRef\]](#)
50. Li, L.; Chen, L.; Zhang, W.; Liao, Y.; Chen, J.; Shi, Y.; Luo, S. Serum cytokine profile in patients with breast cancer. *Cytokine* **2017**, *89*, 173–178. [\[CrossRef\]](#)
51. Pierce, B.L.; Ballard-Barbash, R.; Bernstein, L.; Baumgartner, R.N.; Neuhausser, M.L.; Wener, M.H.; Baumgartner, K.B.; Gilliland, F.D.; Sorensen, B.E.; McTiernan, A.; et al. Elevated biomarkers of inflammation are associated with reduced survival among breast cancer patients. *J. Clin. Oncol. Off. J. Am. Soc. Clin. Oncol.* **2009**, *27*, 3437–3444. [\[CrossRef\]](#) [\[PubMed\]](#)
52. Galluzzi, L.; Senovilla, L.; Zitvogel, L.; Kroemer, G. The secret ally: Immunostimulation by anticancer drugs. *Nat. Rev. Drug Discov.* **2012**, *11*, 215–233. [\[CrossRef\]](#) [\[PubMed\]](#)

53. Wu, J.; Meng, F.; Kong, L.-Y.; Peng, Z.; Ying, Y.; Bornmann, W.G.; Darnay, B.G.; Lamothe, B.; Sun, H.; Talpaz, M.; et al. Association between imatinib-resistant BCR-ABL mutation-negative leukemia and persistent activation of LYN kinase. *J. Natl. Cancer Inst.* **2008**, *100*, 926–939. [[CrossRef](#)] [[PubMed](#)]
54. Choi, Y.-L.; Bocanegra, M.; Kwon, M.J.; Shin, Y.K.; Nam, S.J.; Yang, J.-H.; Kao, J.; Godwin, A.K.; Pollack, J.R. LYN is a mediator of epithelial-mesenchymal transition and a target of dasatinib in breast cancer. *Cancer Res.* **2010**, *70*, 2296–2306. [[CrossRef](#)] [[PubMed](#)]
55. Stein, A.F.V.; Rebollido-Rios, R.; Lukas, A.; Koch, M.; von Lom, A.; Reinartz, S.; Bachurski, D.; Rose, F.; Bozek, K.; Abdallah, A.T.; et al. LYN kinase programs stromal fibroblasts to facilitate leukemic survival via regulation of c-JUN and THBS1. *Nat. Commun.* **2023**, *14*, 1330. [[CrossRef](#)]
56. Dou, T.; Fu, M.; Wang, Y.; Zhao, Y.; Wang, Z.; Bian, Z.; Zhou, Y. Signatures of positive selection in LY96 gene in vertebrates. *J. Biosci.* **2013**, *38*, 899–904. [[CrossRef](#)]
57. Bist, P.; Shu, S.; Lee, H.; Arora, S.; Nair, S.; Lim, J.Y.; Dayalan, J.; Gasser, S.; Biswas, S.K.; Fairhurst, A.-M.; et al. Annexin-A1 regulates TLR-mediated IFN- β production through an interaction with TANK-binding kinase 1. *J. Immunol.* **2013**, *191*, 4375–4382. [[CrossRef](#)]
58. van den Ende, N.S.; Nguyen, A.H.; Jager, A.; Kok, M.; Debets, R.; van Deurzen, C.H.M. Triple-Negative Breast Cancer and Predictive Markers of Response to Neoadjuvant Chemotherapy: A Systematic Review. *Int. J. Mol. Sci.* **2023**, *24*, 2969. [[CrossRef](#)]
59. Li, Y.; Zhang, H.; Merkhher, Y.; Chen, L.; Liu, N.; Leonov, S.; Chen, Y. Recent advances in therapeutic strategies for triple-negative breast cancer. *J. Hematol. Oncol.* **2022**, *15*, 121. [[CrossRef](#)]
60. Ciocan-Cartita, C.A.; Jurj, A.; Zanoaga, O.; Cojocneanu, R.; Pop, L.A.; Moldovan, A.; Moldovan, C.; Zimta, A.A.; Raduly, L.; Pop-Bica, C.; et al. New insights in gene expression alteration as effect of doxorubicin drug resistance in triple negative breast cancer cells. *J. Exp. Clin. Cancer Res.* **2020**, *39*, 241. [[CrossRef](#)]
61. Chou, C.W.; Huang, Y.M.; Chang, Y.J.; Huang, C.Y.; Hung, C.S. Identified the novel resistant biomarkers for taxane-based therapy for triple-negative breast cancer. *Int. J. Med. Sci.* **2021**, *18*, 2521–2531. [[CrossRef](#)] [[PubMed](#)]
62. Christowitz, C.; Davis, T.; Isaacs, A.; van Niekerk, G.; Hattingh, S.; Engelbrecht, A.-M. Mechanisms of doxorubicin-induced drug resistance and drug resistant tumour growth in a murine breast tumour model. *BMC Cancer* **2019**, *19*, 757. [[CrossRef](#)] [[PubMed](#)]
63. Gautier, L.; Møller, M.; Friis-Hansen, L.; Knudsen, S. Alternative mapping of probes to genes for Affymetrix chips. *BMC Bioinform.* **2004**, *5*, 111. [[CrossRef](#)] [[PubMed](#)]
64. Li, Q.; Birkbak, N.J.; Györfy, B.; Szallasi, Z.; Eklund, A.C. Jetset: Selecting the optimal microarray probe set to represent a gene. *BMC Bioinform.* **2011**, *12*, 474. [[CrossRef](#)]
65. Menyhart, O.; Wetz, B.; Györfy, B. MultipleTesting.com: A tool for life science researchers for multiple hypothesis testing correction. *PLoS ONE* **2021**, *16*, e0245824. [[CrossRef](#)]
66. Huber, W.; Carey, V.J.; Gentleman, R.; Anders, S.; Carlson, M.; Carvalho, B.S.; Bravo, H.C.; Davis, S.; Gatto, L.; Girke, T.; et al. Orchestrating high-throughput genomic analysis with Bioconductor. *Nat. Methods* **2015**, *12*, 115–121. [[CrossRef](#)]
67. Sherman, B.T.; Hao, M.; Qiu, J.; Jiao, X.; Baseler, M.W.; Lane, H.C.; Imamichi, T.; Chang, W. DAVID: A web server for functional enrichment analysis and functional annotation of gene lists (2021 update). *Nucleic Acids Res.* **2022**, *50*, W216–W221. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.