Journal of Pioneering Medical Sciences
Received: July 05, 2025 | Accepted: August 08, 2025 | Published: September 05, 2025
Volume 14, Issue 08, Pages 90-99

DOI https://doi.org/10.47310/jpms2025140812

Association of p21waf1 CDK-interacting Protein 1 Expression on Progression Free Survival and TNM Staging in Breast Cancer Patients Using Tamoxifen Hormonal Therapy

Abdulameer Kareem Leelo Al-Obaidy[1*], Hawraa A. Kareem[2] and Yusra Jabbar Hasan[3]

[1]Department of Basic Medical Science, Nursing College, Al-Qadisiyah University, Diwaniyah, Iraq
[2]Department of Al-Jawad Oncology Center, Al-Emamain Al-Kadimain Medical City, Ministry of Health of Iraq, Baghdad, Iraq
[3]Egyptian Board of Medical Oncology, Department of Al-Jawad Oncology Center, Al-Emamain Al-Kadimain Medical City, Baghdad, Iraq

Author Designation: [1]Assistant Professor

*Corresponding author: Abdulameer Kareem Leelo Al-Obaidy (e-mail: Abdulameer.leelo@qu.edu.iq).

Abstract **Objectives: Background and Aim:** Examining the relationship among p21WAF1, Progression-Free Survival (PFS), TNM staging and tamoxifen treatment in hormone-sensitive breast cancer patients may yield insights into additional robust biomarkers for timely identification and prognosis of breast carcinoma. **Patients and Methods:** A prospective observational study was conducted including 78 female patients with breast cancer who were monitored for five years. The mean age among participants was 41.54 years and their mean weight was 75.62 kg prior to therapy. Patients receiving tamoxifen therapy were categorized into four groups based on TNM staging: T2N0M0 (30 patients), T2N1M0 (28 patients), T3N0M0 (14 patients) and the last group T4N1M0 (6 patients). **Results:** All patients are hormone receptor-positive and exhibited varying degrees of p21Waf1 expression, with nuclear p21 expression percentages ranging from 25% to less than 85%. The statistical analysis revealed a substantial reduction in p21Waf1 expression associated with increased tumor growth and lymphatic metastasis (TNM), with $p<0.005$. The influence of TNM staging in conjunction with p21Waf1 expression on Progression-Free Survival (PFS) duration indicates that patients classified as T3N0M0 have the shortest PFS, whereas an increased PFS is noted in T2N1M0, with further enhancement in T4N1M0 and T2N0M0 stages. Patient age and weight prior to treatment significantly impact Progression-Free Survival (PFS) in stages T2N0M0 and T2N1M0 more than in stages T3N0M0 and T4N1M0. **Conclusion:** The findings indicate that p21Waf1 is a crucial regulator of tumor response to tamoxifen and enhancing p21Waf1 levels may be effective in addressing acquired resistance.

Key Words p21WAF1, Breast Cancer, Tamoxifen, Progression Free Survival (PFS), Immunohistochemistry

INTRODUCTION

Tamoxifen in this study is given as adjuvant therapy to prevent tumor recurrence and to increase sensitivity to anti-estrogenic drugs. The follow-up includes monitoring clinical signs, especially in terms of tumor recurrence or cessation. The patients are assessed periodically to distinguish local or distant regrowth of Breast Cancer (BC) on the same side or in the other side of BC, as well as tumor response to endocrine treatment outcomes obtained using imaging techniques, such as nuclear medicine and variations in magnetic resonance imaging according to the oncology doctor's advice. Breast cancer is the most common tumor among women [1,2]. Hormone receptors are nuclear transcription factors that govern cellular growth, regulation and differentiation processes. Tamoxifen is a selective estrogen receptor modulator used in both premenopausal and postmenopausal situations. Upon interacting with the receptor, it alters co-receptor binding and thus modulates gene expression. It opposes the effects of estrogen in breast tissue. Consequently, tamoxifen offers protection against recurrences and prolonged tamoxifen treatment promotes survival in patients with ER-positive breast cancer [3,4]. The p21Waf1 is a Cyclin-Dependent Kinase (CDK) inhibitor that binds to and suppresses the activity of cyclin-CDK2 and CDK1 complexes. It inhibits cells from entering the S phase and developmentally postpones cells that are transiently exiting the cell cycle. Numerous studies in the literature indicate that cancer patients with positive p21Waf1 expression have significantly higher life durations than those with negative p21Waf1 expression. The molecule p21Waf1,

controlled by the hormone receptor pathway, is a CDK-interacting protein essential for cell cycle regulation and proliferation, making it a significant anti-cancer target in hormone-positive breast tumors. It is pivotal in apoptosis, as demonstrated by the research referenced in the review [5-7]. The predominant preoperative staging approach for breast cancer patients is the TNM system, which was established by the American Joint Committee on Cancer and the Union for International Cancer Control. The Tumor Node Metastases (TNM) system is now the most prevalent, dependable and standardized method for classifying tumors and metastases in breast cancer patients [8,9]. Hormone receptors are nuclear transcription factors that regulate cellular growth, regulation and differentiation mechanisms. One such molecule influenced by the hormone receptor pathway is p21Waf1, a CDK-interacting protein with critical functions in cell cycle control and, by extension, cell production, which gains importance as an anti-cancer target in hormone-positive breast tumors. It plays a strategic role in apoptosis, as evidenced in the studies mentioned in the review. Cancer Antigen 15-3 serves as an effective tumor marker for assessing therapeutic efficacy and recurrence in breast cancer. A tumor may lead to metastatic dissemination and typically, a metastatic tumor is associated with worse patient prognosis and reduced survival rates. This study evaluated the expression of p21Waf1 and its correlation with progression-free survival and metastatic BC patients' CA 15-3 values. Findings may enhance treatment responses in breast cancer [10-12].

MATERIELS AND METHODS

Study Design

Seventy-eight breast cancer patients were observed at Al-Emamain Al-Kadimain Medical City/Al-Jawad Oncology Centre or referred to this center from other private oncology hospitals and clinics in Baghdad for more than five years from February 1, 2019, to June 1, 2024. The primary objective of our study was to examine the impact of p21Waf1 protein expression on progression-free survival only in the breast cancer patient group receiving hormonal therapy with tamoxifen. Consequently, p21Waf1 is a prospective predictive target that warrants investigation exclusively in future prospective studies including tamoxifen-treated patients [13-16]. Additionally, we examined the influence of additional factors, including TNM staging, Cancer Antigen (CA 15-3), age and body weight prior to therapy, height and clinical outcomes, in breast cancer patients accurately staged according to the categorization that tackles the illness in this manner [17-20]. All clinical data, including ER, PR, duration of effective endocrine therapy, CA15-3 and PFS, were recorded and collected for analysis from Al-Jawad Oncology Center clinics and laboratory.

Criteria for Patient Eligibility

This research includes 78 participants diagnosed with breast cancer. All patients were female. The inclusion criteria stipulated that (a) participants were over 18 years of age and had a histological diagnosis of BC; (b) patients underwent surgical procedures for the excision of breast cancer tissue; (c) effective endocrine therapy, such as tamoxifen, was administered for a minimum of two years for those included in the study, while other patients were at varying stages of treatment, all remaining in PFS at the conclusion of the study; (d) subsequent hormonal adjuvant therapy was open to pre- and postmenopausal women in whom hormone receptor assays had been carried out on primary tumors; (e) all patients were currently being treated by the same medical oncologist; (f) no other concurrent malignant disease apart from non-melanocytic skin cancer or adequately treated carcinoma in situ of the cervix was allowed if free of disease for more than five years; and (g) comprehensive clinical data for all patients were accessible to oncologists for determining treatment indications, encompassing Estrogen Receptor (ER) and Progesterone Receptor (PR) status, human epidermal growth factor receptor 2 (HER2) status and CA15-3 levels prior to surgery, as well as mean levels during the PFS period, with clinical and investigative follow-ups occurring every three to six months at the oncologist's discretion (21,22,23]. The multipectoral tumor database was utilized to gather the following specific information: menopausal group, TNM stage, nodal status, tumor size, age at initial breast cancer diagnosis, height and patient weight prior to treatment, weight subsequent to treatment. The average assessment of CA15-3 levels was documented following treatment intolerance or tumor advancement [24,25].

Exclusion Criteria were as follows:

- Pregnant or lactating women were disqualified
- Systemic cytotoxic chemotherapy or any other treatment not associated with breast cancer, including radiation and bisphosphonates, administered within 4 weeks prior to study enrollment
- Patients presenting with central nervous system metastases
- Previous history of hepatic metastases
- A previous history of significant medical or psychiatric issues that may impede the patient from fulfilling the research requirements
- Chronic significant immunodeficiency arising from disease, concomitant illness, or pharmacological agents that impair the immune system
- Notable surgical procedures conducted during the 28 days prior to trial involvement
- Uncontrollable active infection [26-29]

Data Collection Methods

This mixed-methods study is designed to provide comprehensive information about our breast cancer patient cohort during follow-up under Tamoxifen therapy. The Coordinating Center is responsible for the quality assurance of the clinical data as well as acquisition, electronic documentation and daily monitoring of all clinical data [30-32]. Al-Jawad Oncology Center records clinical data, laboratory results and

patient-reported outcomes. For diagnostic instruments, treatment delivery, as well as treatment effects, diagnostic and unawareness bias will also be systematically documented [33]. The measurements of p21Waf1 expression were assessed and inputted into a statistical model [34].

Quantitative Data Assessment and Survival Statistical Analysis

A thorough review of all employed methods that leverage bidirectional processes. Linear regression models were constructed within the competing-risk framework to assess the influence of p21Waf1 expression, employing the appropriate threshold, namely the median of the total cohort or the 25th percentile in progression-free survival, in conjunction with fluctuations in CA 15-3 levels. The significance of the time-dependent covariate was evaluated by contrasting the model that incorporates the variable with a model that omits it [35,36]. The clinical data of seventy-eight eligible patients are continuously analyzed to identify statistical trends. The statistical technique utilized to estimate the influence of p21Waf1 expression on PFS in breast cancer is the Kaplan-Meier survival analysis, augmented by the log-rank test for comparing survival curves among different groups, such as patients with high p21Waf1 expression versus those with low or absent p21Waf1 expression. Cox proportional hazards regression models were utilized to assess the impact of p21Waf1 expression on progression-free survival, both alone and in conjunction with TNM staging or other variables. Continuous variable data are presented as means accompanied by interquartile ranges. Hazard ratios and odds ratios are derived from univariable and multivariate analysis. Cox and logistic regression analyses utilized Omnibus Tests of Model Coefficients toassess the time-dependent impacts of variables related to progression-free survival and hormone therapy response rate, as established by Kaplan-Meier survival analysis, Cox Regression, Log Rank (Mantel-Cox), Breslow (Generalised Wilcoxon) and Tarone-Ware tests. The threshold for PFS was determined by Receiver Operating Characteristic (ROC) curve analysis for analytical objectives [37-40]. Survival Analysis Data was retrieved, quality qualification was assessed and any outliers were found through the use of box plots and histograms for p21Waf1 expression across all patients. Potential biases and confounding variables, including the classification of therapy as palliative or adjuvant, were mitigated by assessing these aspects solely in the preoperative context; all patients were administered tamoxifen as the first treatment. A study of the residuals based on the Cox model invalidated the normalcy of progression-free survival, leading us to use the fifth percentile as the cutoff for PFS, establishing 22 months as the threshold for PFS. Consequently, increases in p21Waf1 CDK-interacting protein 1 suggest a reduction in mortality risk [41-43]. Based on the patients' TNM staging, we categorized those receiving tamoxifen therapies into four groups: the first group T2N0M0, the second group T2N1M0, the third group T3N0M0 and the fourth group T4N1M0 [44,45].

Immunohistochemical Analysis and Scoring Methods

Immunohistochemistry was performed on all 78 breast cancer patients to detect p21Waf1, with a kit from Ventana Medical Systems, Inc., situated at 1910 E. Innovation Park Drive, Tucson, Arizona 85755, USA, in accordance with the manufacturer's standardized protocol. Paraffin-embedded tumor blocks from the patients were sliced to a thickness of 4 µm. Serial dilutions of the p21Waf1 primary monoclonal antibody were conducted at ratios of 1:25, 1:50 and 1:100. The staining intensity remained uniform across the dilutions, resulting in the selection of the 1:100 dilution for the tests, using slide tissue-fixed samples administered with a mouse monoclonal antibody targeting human p21Waf1. The expression of p21Waf1 was visualized using diaminobenzidine tetrahydrochloride (DAB; Sigma, St. Louis, MO) for five minutes. The slides were dehydrated, counterstained with Mayer's haematoxylin and mounted using DePex (BDH, Poole, Dorset, UK). Matching the analyzed slides, each staining series included positive (p21Waf1 positive colorectal carcinoma) and negative (breast cancer without primary antibody) control slides. Only p21Waf1 nuclear staining demonstrated a positive connection when present. The percentage score of p21 expression used for immunostaining was obtained from the tumor's greatest cellular region, distinguished by minimal inflammatory cell infiltration or necrosis and the highest nuclear density. The invasive component cells were included in the statistical analysis, with the count of positively stained nuclei documented at x400 magnification. The proportion of p21 expression in the tumor nuclei was ascertained by counting 1,000 cells per slide. The measurement of the overall percentage excluded cytoplasmic expression. Normal breast tissue seldom exhibited p21 expression, predominantly seen in myoepithelial cells and scattered acinar cells [46,47]. The capability of Immunohistochemistry (IHC) that can be conducted on Formalin-Fixed Paraffin-Embedded (FFPE) tumors is a significant benefit. Various grading systems for p21Waf1 expression have been suggested, including the histology scoring system and semi-quantitative techniques. Each has benefits and limits that render them advantageous in specific circumstances. Typically, the grading considers both the percentage of positively stained tumor cells and the intensity of staining. p21Waf1 is measured as the percentage of tumor cells with nuclear staining:

0 = 1 to 25%; 1 = 26 to 50%; 2 = 51 to 75%; 3 = 76 to 100%

RESULTS

This study sought to determine the relationship between p21Waf1 (cyclin-dependent kinase inhibitor) expression and Progression-Free Survival, considering the influence of TNM (tumor, node, metastasis) staging of breast cancer, as derived from patient records and surgical pathology results obtained from the professionals' database, along with the impact of other variables such as mean Cancer Antigen (CA 15-1) levels during the PFS period, as well as the age and weight of patients.

Table 1: Shows the Primary Variable Frequencies Included in the Research

Parameters	Age of Patients	Height of Patients	Weight before Rx	Weight after Rx	Ca.15.3 Before Rx	CA.15.3 after Rx	PFS Period	p21Waf1
Number Patients	78	78	78	78	78	78	78	78
Mean	41.54	160.54	75.62	81.77	45.69	10.46	43.95	68.8%
Median	46.00	162.00	77.00	83.00	37.00	10.00	47.00	68%
Std. Deviation	9.314	6.225	9.512	9.783	21.97	6.224	13.26	13.4%
Minimum	25	145	55	62	4	1	24.00	45%
Maximum	57	168	90	99	90	23	69.00	89%

Table 2: Indicates the Distribution of Carcinoma of the Breast Patients by Tumor TNM Staging and their Corresponding Percentages

TNM staging	Frequency	Percent	Valid Percent	Cumulative Percent
T2N0M0	30	38.5	38.5	38.5
T2N1M0	28	35.9	35.9	74.4
T3N0M0	14	17.9	17.9	92.3
T4N1M0	6	7.7	7.7	100
Total	78	100	100	-

Table 3: Evaluation of the equivalence of survival disseminations at different levels of p21 expression in relation to TNM staging

Parameters	Mean	T2N0M0	T2N1M0	T3N0M0	T4N1M0
p21 expression	68.769	68.769	68.769	68.769	68.769
T2N0M0	0.385	1.000	0.000	0.000	0.000
T2N1M0	0.359	0.000	1.000	0.000	0.000
T3N0M0	0.179	0.000	0.000	1.000	0.000
Age of Patients	41.538	41.538	41.538	41.538	41.538

In accordance with the data presented in Table 1, each of the seventy-eight patients was a female and their average age was 41.54 years, while the median age was 46.00 years. All of the patients had a mean weight of 75.62 kg prior to beginning treatment.

The means for all patients are as follows: the Progression-Free Survival (PFS) time was 43.95 months, p21Waf1 expression was 68.8% and the Cancer Antigen CA 15-1 level post-treatment was 10.46 Unit/ml, along with further means presented in Table 1.

TNM staging is the primary criterion for assessing tumor malignancy in patients, determined by the tumor's size, location and metastasis. Based on the patients' TNM staging, we categorized that receiving tamoxifen therapy into four groups: the first group T2N0M0 (30 patients, 38.5%), the second group T2N1M0 (28 patients, 35.9%), the third group T3N0M0 (14 patients, 17.9%) and the final group T4N1M0 (6 patients, 7.7%) as presented in Table 2.

Immunohistochemical analysis of paraffin-embedded breast cancer tissue revealed a mean p21Waf1 protein expression of 68.7% among tumor cells, with variability based on TNM staging, as presented in Table 3. All 78 breast cancer patients had varying degrees of positive p21Waf1 expression in their tissue samples, with nuclear p21Waf1 expression percentages ranging from 25% to less than 85%. The statistical analysis indicated no significant difference in p21Waf1 expression concerning age ($p>0.05$). In contrast, a noteworthy distinction was seen in the expression of p21Waf1 in relation to the size of the tumor and the presence of Lymphatic Metastasis (TNM), with a p-value of less than 0.005. This indicates that there is a drop in p21Waf1 expression that is related to higher TNM stages, as shown in Table 3.

Following five years of monitoring patients undergoing tamoxifen treatment, routine follow-up visits occur every three months during the initial three years post-treatment and then every six months in the succeeding years. The frequency of visits was adjusted according to the risk of relapse and patient requirements [22,31]; all patients remained free of metastases at the conclusion of the follow up period.

Examination of Survival Data for patients regarding p21Waf1 expression and other variables demonstrates a substantial relationship between their expression and PFS periods with a p value less than 0.0005, as seen in Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon) and Tarone-Ware, indicating that a larger PFS period corresponds with a higher percentage of p21Waf1 expression in breast cancer tissue. Upon examining the impact of TNM staging alongside p21Waf1 expression and Progression-Free Survival (PFS) duration through Cox regression analysis, it is evident that patients in stage T3N0M0 exhibit the shortest PFS, with an increase observed in stage T2N1M0 and a further increase in stages T4N1M0 and T2N0M0 (Figure 1). However, when additional variables, such as patient age, are incorporated into the analysis, a notable enhancement in PFS is observed in stage T2N0M0 compared to stage T4N1M0, while stages T3N0M0 and T2N1M0 continue to demonstrate lower PFS (Figure 2). The weight of patients prior to therapy significantly influences Progression-Free Survival (PFS) in stages T2N0M0 and T2N1M0 more than in stages T3N0M0 and T4N1M0 (Figure 3).

There is no substantial correlation between Progression-Free Survival (PFS) or TNM staging and the CA 15-1 protein during the treatment period, including the patients' height. However, a significant relationship exists between CA 15-1 levels prior to treatment and surgical intervention concerning

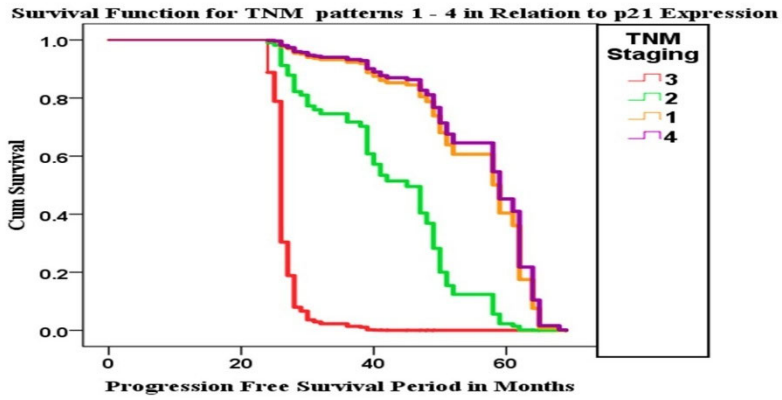

Figure 1: Illustrate the influence of p21Waf1 expression and TNM staging on the patients progression-free period durations in months

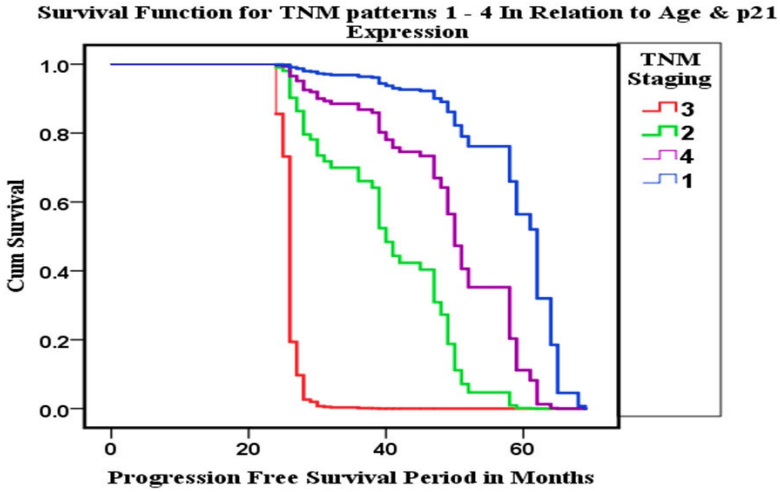

Figure 2: Demonstrate the Impact of Patient Age on Progression-Free Survival Periods in Relation to TNM Staging and p21Waf1 Expression

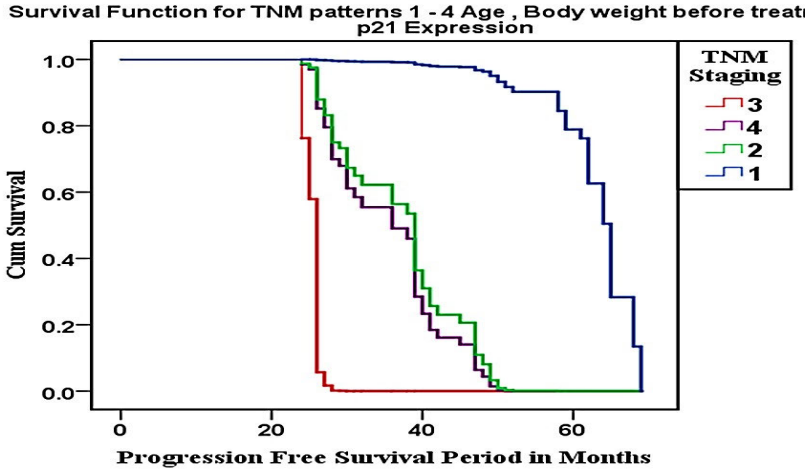

Figure 3: Distribution of patients based on the impact of TNM staging, p21Waf1 expression, age, and patient weight prior to therapy on the progression-free survival duration in months

TNM staging, as evidenced by Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon) and Tarone-Ware tests, all yielding a P value below 0.0005. Furthermore, CA 15-1 levels increase in accordance with the advancement of the patient's metastasis stage [34,49].

DISCUSSION

The relationship between p21 Waf1 CDK-interacting protein (p21) expression and the clinical response to hormone treatments such as Tamoxifen is complex. It has been demonstrated through research that its expression is a predictor of enhanced PFS as well as overall survival, while others have found it to be independent of patient outcome [48,50]. These conflicting findings can be attributed to the exact role p21 Waf1 has, being dependent on the cell cycle phase it encounters. It is known to arrest post-mitotic cells in the G1 phase of the cell cycle and can lead to increases in apoptosis in laboratories. It is essential to conduct an analysis of the expression of p21 Waf1 because there is a growing controversy concerning the molecular action of Tamoxifen and the patients who are able to reap the benefits of this treatment [51-54].

The findings of the current research suggest that the expression of p21Waf1 is strongly associated with the advancement of BC as well as the spread of the disease to other parts of the body. It also suggests that p21 can serve as a clinical indicator for breast cancer, particularly in combination with other markers. Survival Analysis Data concerning p21Waf1 expression and additional variables reveal a strong association between their expression and PFS durations, with a P value below 0.0005, as evidenced by Log Rank (Mantel-Cox), Breslow (Generalized Wilcoxon) and Tarone-Ware tests, suggesting that an extended PFS duration is associated with an increased percentage of p21Waf1 expression in breast cancer tissue. Analysis of the influence of TNM staging in conjunction with p21Waf1 expression and Progression-Free Survival (PFS) duration via Cox regression reveals that patients classified as T3N0M0 experience the shortest PFS, with an improvement noted in T2N1M0 and a further enhancement in T4N1M0 and T2N0M0 stages (refer to Figure 1). Nevertheless, when supplementary factors, such as patient age, are included into the study, a substantial enhancement in PFS is shown in stage T2N0M0 relative to stage T4N1M0, although stages T3N0M0 and T2N1M0 persist in exhibiting worse PFS (refer to Figure 2). Patient weight prior to treatment markedly affects Progression-Free Survival (PFS) in stages T2N0M0 and T2N1M0 more than in stages T3N0M0 and T4N1M0. Although we anticipate that T4N1M0 represents the shortest Progression-Free Survival (PFS) duration, this can be attributed to the limited patient population at this stage. Additionally, patient age, as illustrated in Figure 2 and patient weight, as depicted in Figure 3, also influenced the PFS duration.

The statistical test indicates an important association between p21Waf1 and clinical stage, as well as TNM expression ranks, suggesting p21Waf1's involvement in tumor growth. With the TNM score and clinical stage increased, the expression level of p21Waf1 decreases, suggesting that p21Waf1 is closely related to invasion promotion for breast cancer. A number of additional studies, including those conducted by other researchers [45,50,55,56], support this finding. Multinomial logistic regression results also indicate that the combined factors of clinical stage, p21Waf1 and TNM are statistically significant in forecasting the prognosis of breast cancer. Therefore, the combined application of p21 and TNM for predicting prognosis in BC may provide a novel basis for clinical diagnosis and treatment. All patients, after more than four years of follow-up, still have a good response to tamoxifen adjuvant therapy, indicating the role of p21 as a biomarker for a good response. In a series of studies on human breast cancer cell lines, adenoviral-expressed p21 was found to sensitize cells to tamoxifen in a dose-dependent manner. Conversely, cultures with lower p21 expression were resistant to this effect. Further examination indicated that p21-enhanced tamoxifen coupling to ER occurred through a cellular event involving p21-induced G1 phase arrest and resultant re-localization of a pool of intracellular ER to the nucleus, permitting enhanced antiestrogen action. Together, these results suggest that p21 status is a significant modulator of tumor response to tamoxifen and that strategies to augment p21 levels may be fruitful in overcoming acquired resistance. Tamoxifen is an estrogen analogue that binds to the Estrogen Receptor (ER) and inhibits estrogen-induced breast cancer growth. However, approximately one-third of tamoxifen-treated patients develop a recurrence of disease. While the mechanisms underlying acquired resistance remain poorly understood, low levels of the CDK inhibitor p21WAF1/CIP1 have been implicated in antiestrogen resistance [57,58].

Understanding the link between p21, TNM staging and tamoxifen therapy in hormonally sensitive breast cancer patients may lead to more comprehensive biomarkers for early breast cancer diagnosis. The inactivation of p53 and increase of p16Ink4a and p21Cip1/Waf1 promote BC in many steps. TNM serves as a valuable prognostic biomarker for cancer, facilitating treatment planning. Researchers have convened to investigate the expression of p21 in breast cancer. The progression of breast cancer can be anticipated through the TNM score and clinical stage. Breast cancer can initiate, advance and evolve with increasing tumor size, lymph node metastasis and distant metastasis. The CA 15-13 protein, along with patient height, does not influence Progression-Free Survival (PFS) or TNM staging. This is due to all patients being in the PFS period and exhibiting no statistically significant alterations in CA 15-3 levels [59-64].

CONCLUSION

The analysis of the impact of TNM staging alongside p21Waf1 expression on Progression-Free Survival (PFS) duration through Cox regression indicates that patients categorized as T3N0M0 exhibit the shortest PFS, with an enhancement observed in T2N1M0 and a further improvement in T4N1M0 and T2N0M0 stages.

This study indicated that p21Waf1 and its immunohistochemistry expression predict tamoxifen therapy and govern breast cancer treatment resistance; therefore new diagnostic, prognostic and therapeutic targets for customized breast cancer treatment should be studied. It stresses the importance of understanding pathway crosstalk

and investigating if this protein could affect biomarkers in this therapy. More breast cancer patients should be used to confirm the statistical result and p21Waf1 marker's functional roles should be studied. Cancer biomarker reviews are intriguing because they improve treatment. This study predicts patient outcomes.

Acknowledgement
We thank the Al-Jawad Oncology Center (JOC) in Al-Emamain Kadimain Medical City/Baghdad's and its laboratory personnel for their invaluable assistance and involvement during this work. Their expertise, facilities and resources were critical to our research and we appreciate the time and effort they put into our project. This study was made possible by the Al-Jawad Oncology Center and its laboratory and we are grateful for their assistance.

Conflicts of Interest
It has been stated by the authors that there are no potential conflicts of interest associated with this research. They have stated that they have made this declaration. With regard to the planning, execution, analysis and reporting of this study, there have been no influences that have been influential, whether they be financial, personal, or institutional in nature. The authors have stated all of the sources of financing in a manner that is both open and transparent. The authors assert that they have complied with ethical research standards and have disclosed complete information regarding any potential competing interests that could have an impact on the outcomes of the study. In order to maintain both transparency and integrity in the dissemination of research, any potential conflicts of interest that may arise in the future will be declared as soon as feasible. This is done with the intention of adhering to the principles of transparency and integrity.

Ethical Statement
Ethics approval was meticulously followed in all study processes to assure compliance and integrity. The treatment schedule proceeded unaffected by the study's influence on the physicians' decisions; this study primarily relied on tissue samples obtained for breast cancer diagnosis by the oncologist and/or surgeon. Paraffin-embedded tissue samples were sourced from the histopathological laboratory archives to assess p21Waf1 expression after patient diagnosis and staging. The oncology council developed the treatment course without regard for the relevant research on the treatment protocol. Patients received hormonal treatment for breast cancer as determined by the oncologists at the Al-Jawad Oncology Centre and any necessary assessment of p21Waf1 expression was conducted independently of this research. The cost of the study and immunohistochemistry test was determined by the study and was derived from the study expenses, with no relation to the patients. Ethical consent was secured from the Al-Jawad Oncology Centre and other private institutions where the histopathological diagnosis was performed. The study was carried out according to the Helsinki Declaration and good clinical practice guidelines, having been authorized by the Institutional Review Board and Ethical Committee of Al-Jawad Oncology Center. All patients provided informed permission prior to enrollment in therapy by the oncology staff.

Author Contribution
All contributors were involved in the conceptualization and helped secure financial assistance. All study expenses, including the immunohistochemistry test and associated charges, were covered by the researcher rather than the patients. Dr. Hawraa A. Kareem and Dr. Yusra Jabbar Hasan, both Senior Oncologists at Al-Jawad Oncology Center in Al-Emamain Kadimain Medical City/Baghdad, conducted a clinical evaluation of the patient, diagnosed PFS and provided continued oversight. Dr. Abdulameer Kareem's preliminary paper included immunohistochemical analysis, statistical data analysis, composition and supervision. Leelo Al-Obaidy, M.B.Ch.B., Ph.D., specializes in clinical immunology.

REFERENCES

[1] Mahadev, B. *et al.* "Effect of tamoxifen on plasma lipid profile in patients of breast cancer." *Journal of Ayub Medical College Abbottabad,* vol. 35, no. 4, October 2023, pp. 558-562. http://dx.doi.org/10.55519/jamc-04-7330.

[2] Kumar, S. *et al.* "Hormonal and targeted treatments in breast cancer." In Breast Cancer: Comprehensive Management, pp. 443-463. Singapore: Springer Nature Singapore, 2022.

[3] Ghanavati, M. *et al.* "Tamoxifen use and risk of endometrial cancer in breast cancer patients: A systematic review and dose–response meta-analysis." *Cancer Reports,* vol. 6, no. 4, March 2023, pp. e1806. http://dx.doi.org/10.1002/cnr2.1806.

[4] Remmel, H.L. *et al.* "Comparative analysis of Endoxifen, Tamoxifen and Fulvestrant: A Bioinformatics Approach to Uncover Mechanisms of Action in Breast Cancer." bioRxiv, pp. 2024-10. 2024, biorxiv.org.

[5] Falato, C. *et al.* "Clinical implications of the intrinsic molecular subtypes in hormone receptor-positive and her2-negative metastatic breast cancer." *Cancer Treatment Reviews,* vol. 112, January 2023, pp. 102496. http://dx.doi.org/10.1016/j.ctrv.2022.102496.

[6] Satpathi, S. *et al.* "Unveiling the role of hormonal imbalance in breast cancer development: A comprehensive review." *Cureus,* vol. 15, no. 7, July 2023, pp. e41737-0. http://dx.doi.org/10.7759/cureus.41737.

[7] Smolarz, B. *et al.* "Breast cancer-epidemiology, classification, pathogenesis and treatment (review of literature)." *Cancers,* vol. 14, no. 10, May 2022, pp. 2569-0. http://dx.doi.org/10.3390/cancers14102569.

[8] Lim, S. *et al.* "Anticancer effect of e26 transformation-specific homologous factor through the induction of senescence and the inhibition of epithelial–mesenchymal transition in triple-negative breast cancer cells." *Cancers,* vol. 15, no. 21, November 2023, pp. 5270-0. http://dx.doi.org/10.3390/cancers15215270.

[9] Tsai, Y.C. "Investigating radiation therapy treatments with cell cycle and DNA repair inhibitors in triple-negative breast cancer." Department of Medicine, McGill University, 2024. https://escholarship.mcgill.ca/concern/theses/4x51hq954

[10] Liao, Y. *et al.* "Ethyl acetate extract of antenoron filiforme inhibits the proliferation of triple negative breast cancer cells via suppressing skp2/p21 signaling axis." *Phytomedicine,* vol. 116, July 2023, pp. 154856-0. http://dx.doi.org/10.1016/j.phymed.2023.154856.

[11] Shaikh, M.A.J. *et al.* "Non-coding RNAs: Key regulators of CDK and Wnt/β-catenin signaling in cancer." *Pathology - Research and Practice,* vol. 263, November 2024, pp. 155659-0. http://dx.doi.org/10.1016/j.prp.2024.155659.

[12] Gil-Gas, C. *et al.* "Self-renewal inhibition in breast cancer stem cells: Moonlight role of PEDF in breast cancer." *Cancers,* vol. 15, no. 22, November 2023, pp. 5422-0. http://dx.doi.org/10.3390/cancers15225422.

[13] Cheng, W.Y. *et al.* "Polymorphism at codon 31 of CDKN1A (p21) as a predictive factor for bevacizumab therapy in glioblastoma multiforme." *BMC Cancer,* vol. 23, no. 1, September 2023, pp. 886-0. http://dx.doi.org/10.1186/s12885-023-11400-5.

[14] Aung, T.M. *et al.* "Biomarkers for prognosis of meningioma patients: A systematic review and meta-analysis." *PLOS ONE,* vol. 19, no. 5, May 2024, pp. e0303337-0. http://dx.doi.org/10.1371/journal.pone.0303337.

[15] Wang, Y. *et al.* "Prognostic value of matrix metalloproteinase-2 protein and matrix metalloproteinase-9 protein in colorectal cancer: A meta-analysis." *BMC Cancer,* vol. 24, no. 1, August 2024, pp. 1065-0. http://dx.doi.org/10.1186/s12885-024-12775-9.

[16] Sun, J. *et al.* "Butyrate as a promising therapeutic target in cancer: From pathogenesis to clinic (review)." *International Journal of Oncology,* vol. 64, no. 4, February 2024, pp. 44-0. http://dx.doi.org/10.3892/ijo.2024.5632

[17] Höller, A. *et al.* ""Diagnostic and prognostic biomarkers of luminal breast cancer: Where are we now?" *Breast Cancer: Targets and Therapy,* vol. 15, July 2023, pp. 525-540. http://dx.doi.org/10.2147/bctt.s340741.

[18] Khallouki, F. *et al.* "An update on tamoxifen and the chemo-preventive potential of vitamin e in breast cancer management." *Journal of Personalized Medicine,* vol. 13, no. 5, April 2023, pp. 754-0. http://dx.doi.org/10.3390/jpm13050754.

[19] Mir, Manzoor Ahmad, Aabida Gul, Shazia Sofi and M. Sultan Khan. "CDk Inhibitor for Treatment of Breast Cancer." In: Therapeutic Potential of Cell Cycle Kinases in Breast Cancer, Singapore: Springer Nature Singapore, 2023, pp. 313-333.

[20] Cheng, X. *et al.* "Breast cancer mutations HER2V777L and PIK3CAH1047R activate the p21-CDK4/6-Cyclin D1 axis to drive tumorigenesis and drug resistance." *Cancer Research,* vol. 83, no. 17, June 2023, pp. 2839-2857. http://dx.doi.org/10.1158/0008-5472.can-22-3558.

[21] Harris, M.A. *et al.* "Towards targeting the breast cancer immune microenvironment." *Nature Reviews Cancer,* vol. 24, no. 8, July 2024, pp. 554-577. http://dx.doi.org/10.1038/s41568-024-00714-6.

[22] W Wiecken, M. *et al.* "The immune checkpoint lag-3 is expressed by melanoma cells and correlates with clinical progression of the melanoma." *OncoImmunology,* vol. 14, no. 1, December 2025, pp. 2430066-0. http://dx.doi.org/10.1080/2162402x.2024.2430066.

[23] Yücel, K.B. *et al.* "Visceral obesity and sarcopenia as predictors of efficacy and hematological toxicity in patients with metastatic breast cancer treated with CDK 4/6 inhibitors." *Cancer Chemotherapy and Pharmacology,* vol. 93, no. 5, March 2024, pp. 497-507. http://dx.doi.org/10.1007/s00280-024-04641-z.

[24] Hutajulu, S.H. *et al.* "Delays in the presentation and diagnosis of women with breast cancer in yogyakarta, Indonesia: A retrospective observational study." *PLOS ONE,* vol. 17, no. 1, January 2022, pp. e0262468-0. http://dx.doi.org/10.1371/journal.pone.0262468.

[25] Backhaus, P. *et al.* "Simultaneous FAPI PET/MRI targeting the fibroblast-activation protein for breast cancer." *Radiology,* vol. 302, no. 1, January 2022, pp. 39-47. http://dx.doi.org/10.1148/radiol.2021204677.

[26] Vitale, S.G. *et al.* "Risk of endometrial malignancy in women treated for breast cancer: The BLUSH prediction model - evidence from a comprehensive multicentric retrospective cohort study." *Climacteric,* vol. 27, no. 5, July 2024, pp. 482-488. http://dx.doi.org/10.1080/13697137.2024.2376189.

[27] Brunner, C. *et al.* "Incidence of medication-related osteonecrosis of the jaw in patients with breast cancer during a 20-year follow-up: A population-based multicenter retrospective study." *Journal of Clinical Oncology,* vol. 43, no. 2, January 2025, pp. 180-188. http://dx.doi.org/10.1200/jco.24.00171.

[28] Faleh, S. *et al.* "Predictors of nodal metastases in early stage her2+ breast cancer: Deciding on treatment approach with neoadjuvant chemotherapy vs. upfront surgery." *European Journal of Surgical Oncology,* vol. 49, no. 8, August 2023, pp. 1411-1416. http://dx.doi.org/10.1016/j.ejso.2023.03.226.

[29] Yin, W. *et al.* "Neoadjuvant trastuzumab and Pyrotinib for locally advanced HER2-positive breast cancer (NeoATP): Primary analysis of a phase II study." *Clinical Cancer Research,* vol. 28, no. 17, June 2022, pp. 3677-3685. http://dx.doi.org/10.1158/1078-0432.ccr-22-0446.

[30] Chakravarti, N. *et al.* "XPO1 blockade with KPT-330 promotes apoptosis in cutaneous T-cell lymphoma by activating the p53-p21 and p27 pathways." *Scientific Reports,* vol. 14, no. 1, April 2024, pp. 9305-0. http://dx.doi.org/10.1038/s41598-024-59994-5.

[31] Li, C. *et al.* "The role of cornulin (CRNN) in the progression of cutaneous squamous cell carcinoma involving AKT activation in SCL-1." *PLOS ONE,* vol. 19, no. 9, September 2024, pp. e0308243-0. http://dx.doi.org/10.1371/journal.pone.0308243.

[32] Zheng, H. *et al.* "MAP4K4 and WT1 mediate SOX6-induced cellular senescence by synergistically activating the ATF2-TGFβ2-Smad2/3 signaling pathway in cervical cancer." *Molecular Oncology,* vol. 18, no. 5, February 2024, pp. 1327-1346. http://dx.doi.org/10.1002/1878-0261.13613.

[33] Morgan, J. *et al.* "Psychosocial outcomes after varying risk management strategies in women at increased familial breast cancer risk: A mixed methods study of patient and partner outcomes." *The Annals of The Royal College of Surgeons of England,* vol. 106, no. 1, January 2024, pp. 78-91. http://dx.doi.org/10.1308/rcsann.2023.0042.

[34] Elfoly, M. *et al.* "Pd-l1 intrinsically promotes the proliferation of breast cancer cells through the skp2-p27/p21 axis." *Cancer Cell International,* vol. 24, no. 1, May 2024, pp. 161-0. http://dx.doi.org/10.1186/s12935-024-03354-w.

[35] Yue, Y. *et al.* "N6-methyladenosine-mediated downregulation of mir-374c-5p promotes cadmium-induced cell proliferation and metastasis by targeting grm3 in breast cancer cells." *Ecotoxicology and Environmental Safety,* vol. 229, January 2022, pp. 113085-0. http://dx.doi.org/10.1016/j.ecoenv.2021.113085.

[36] Choi, S. *et al.* "MicroRNA-606 inhibits the growth and metastasis of triple-negative breast cancer by targeting Stanniocalcin 1." *Oncology Reports,* vol. 51, no. 1, November 2023. http://dx.doi.org/10.3892/or.2023.8661.

[37] Nasri, E. *et al.* "Cell Cycle Checkpoints p16 and p21-Strong Predictors of Clinicopathologic Outcomes in High-Grade Osteosarcoma." *The Cancer Journal,* vol. 30, no. 3, May 2024, pp. 133-139. http://dx.doi.org/10.1097/ppo.0000000000000714.

[38] do Nascimento, R.G. *et al.* "Prognostic value of Maspin protein level in patients with triple negative breast cancer." *Scientific Reports,* vol. 14, no. 1, July 2024, pp. 15982-0. http://dx.doi.org/10.1038/s41598-024-53870-y.

[39] Westaby, D. *et al.* "BCL2 expression is enriched in advanced prostate cancer with features of lineage plasticity." *Journal of Clinical Investigation,* vol. 134, no. 18, September 2024, pp. e179998-0. http://dx.doi.org/10.1172/jci179998.

[40] Koh, Y.W. *et al.* "Senescence cell signature associated with poor prognosis, epithelial-mesenchymal transition, solid histology and spread through air spaces in lung adenocarcinoma." *GeroScience,* vol. 47, no. 2, November 2024, pp. 2423-2438. http://dx.doi.org/10.1007/s11357-024-01442-3.

[41] Huang, Y. *et al.* "Cost-effectiveness of adjuvant endocrine treatment with tamoxifen for male breast cancer." *Breast Cancer,* vol. 31, no. 5, June 2024, pp. 917-925. http://dx.doi.org/10.1007/s12282-024-01605-2.

[42] Gowdini, E. *et al.* "DOK7 CpG hypermethylation in blood leukocytes as an epigenetic biomarker for acquired tamoxifen resistant in breast cancer." *Journal of Human Genetics,* vol. 68, no. 1, November 2022, pp. 33-38. http://dx.doi.org/10.1038/s10038-022-01092-3.

[43] Liang, X. *et al.* "DLGAP1-AS2 promotes estrogen receptor signalling and confers tamoxifen resistance in breast cancer." *Molecular Biology Reports,* vol. 49, no. 5, April 2022, pp. 3939-3947. http://dx.doi.org/10.1007/s11033-022-07244-0.

[44] Tian, Y. *et al.* "DHMMF, a natural flavonoid from Resina Draconis, inhibits hepatocellular carcinoma progression via inducing apoptosis and G2/M phase arrest mediated by DNA damage-driven upregulation of p21." *Biochemical Pharmacology,* vol. 211, May 2023, pp. 115518-0. http://dx.doi.org/10.1016/j.bcp.2023.115518.

[45] Nasir, A. *et al.* "p21 Protein Outperforms Clinico-pathological Criteria in Predicting Liver Metastases in Pancreatic Endocrine Tumors." *Cancer Genomics - Proteomics,* vol. 20, no. 6, 2023, pp. 522-530. http://dx.doi.org/10.21873/cgp.20402.

[46] Leelo, A.K. and H.A.K. Leelo. "Immunohistochemical expression of TGF-β1 and Ki-67 in a paraffin-embedded section of breast cancer tissue." *Indian Journal of Forensic Medicine & Toxicology,* vol. 13, no. 4, 2019, pp. 1134-0. http://dx.doi.org/10.5958/0973-9130.2019.00453.5.

[47] Kumar, S. *et al.* "Cyclin-dependent kinase inhibitor p21 and proliferative marker ki67 in colonic carcinoma." *Journal of Cancer Research and Therapeutics,* vol. 18, no. 4, July 2022, pp. 915-920. http://dx.doi.org/10.4103/jcrt.jcrt_712_21.

[48] Rajendran, S. *et al.* "p21 activated kinase-1 and tamoxifen - A deadly nexus impacting breast cancer outcomes." *Biochimica et Biophysica Acta (BBA) - Reviews on Cancer,* vol. 1877, no. 1, January 2022, pp. 188668-0. http://dx.doi.org/10.1016/j.bbcan.2021.188668.

[49] Cutty, S.J. *et al.* "Pro-survival roles for p21(Cip1/Waf1) in non-small cell lung cancer." *British Journal of Cancer,* vol. 132, no. 5, December 2024, pp. 421-437. http://dx.doi.org/10.1038/s41416-024-02928-9.

[50] Manousakis, E. *et al.* "CDKN1A/p21 in Breast Cancer: Part of the Problem, or Part of the Solution?" *International Journal of Molecular Sciences,* vol. 24, no. 24, December 2023, pp. 17488-0. http://dx.doi.org/10.3390/ijms242417488.

[51] White-Gilbertson, S. *et al.* "Transcriptome analysis of polyploid giant cancer cells and their progeny reveals a functional role for p21 in polyploidization and depolyploidization." *Journal of Biological Chemistry,* vol. 300, no. 4, April 2024, pp. 107136-0. http://dx.doi.org/10.1016/j.jbc.2024.107136.

[52] Ooi, L,C. *et al.* "P21 as a predictor and prognostic indicator of clinical outcome in rectal cancer patients." *International Journal of Molecular Sciences,* vol. 25, no. 2, January 2024, pp. 725-0. http://dx.doi.org/10.3390/ijms25020725.

[53] Nakao, T. *et al.* "Prognostic impact of the combination of p16ink4a, p21 and immunoscore in rectal cancer." *International Journal of Clinical Oncology,* vol. 29, no. 8, June 2024, pp. 1152-1160. http://dx.doi.org/10.1007/s10147-024-02519-x.

[54] Celia Gómez de, C. *et al.* "Low-level expression of p-S6 is associated with nodal metastasis in patients with head and neck cutaneous squamous cell carcinoma." *International Journal of Molecular Sciences,* vol. 25, no. 8, April 2024, pp. 4304-0. http://dx.doi.org/10.3390/ijms25084304.

[55] Wang, Y. *et al.* "Improved breast cancer histological grading using deep learning." *Annals of Oncology,* vol. 33, no. 1, January 2022, pp. 89-98. http://dx.doi.org/10.1016/j.annonc.2021.09.007. 55.

[56] Fei, Yang, *et al.* "Clinicopathological significance and prognostic analysis of p21 and EGFR in colorectal cancer: A retrospective analysis on 12 319 cases in China." *J. Clin. Pathol.* June 2024. http://dx.doi.org/10.1136/jcp-2024-209450.

[57] Lee, D.H. *et al.* "CDK4/6 inhibitors induce breast cancer senescence with enhanced anti-tumor immunogenic properties compared with DNA-damaging agents." *Molecular Oncology,* vol. 18, no. 1, November 2023, pp. 216-232. http://dx.doi.org/10.1002/1878-0261.13541.

[58] Melo, M.L. *et al.* "N-acylhydrazone derivative modulates cell cycle regulators promoting mitosis arrest and apoptosis in estrogen positive mcf-7 breast cancer cells." *Toxicology in Vitro,* vol. 93, December 2023, pp. 105686-0. http://dx.doi.org/10.1016/j.tiv.2023.105686.

[59] Margulies, B.S. *et al.* "Reshaping the landscape of locoregional treatments for breast cancer liver metastases: A novel, intratumoral, p21-targeted percutaneous therapy increases survival in balb/c mice inoculated with 4t1 triple negative breast cancer cells in the liver." *PLOS One,* vol. 20, no. 6, June 2025, pp. e0323621-0. http://dx.doi.org/10.1371/journal.pone.0323621.

[60] Chatterji, S. *et al.* "Defining genomic, transcriptomic, proteomic, epigenetic and phenotypic biomarkers with prognostic capability in male breast cancer: A systematic review." *The Lancet Oncology,* vol. 24, no. 2, February 2023, pp. e74-e85. http://dx.doi.org/10.1016/s1470-2045(22)00633-7.

[61] Tarighati, E. *et al.* "A review of prognostic and predictive biomarkers in breast cancer." *Clinical and Experimental Medicine,* vol. 23, no. 1, January 2022, pp. 1-16. http://dx.doi.org/10.1007/s10238-021-00781-1.

[62] Boicean, A. *et al.* "In pursuit of novel markers: Unraveling the potential of mir-106, cea and ca 19-9 in gastric adenocarcinoma diagnosis and staging." *International Journal of Molecular Sciences,* vol. 25, no. 14, July 2024, pp. 7898-0. http://dx.doi.org/10.3390/ijms25147898.

[63] Shen, X. *et al.* "Hsa_circ_0000437 promotes pathogenesis of gastric cancer and lymph node metastasis." *Oncogene,* vol. 41, no. 42, September 2022, pp. 4724-4735. http://dx.doi.org/10.1038/s41388-022-02449-w.

[64] Liu, R. *et al.* "Stnm1 in human cancers: Role, function and potential therapy sensitizer." *Cellular Signalling,* vol. 109, September 2023, pp. 110775-0. http://dx.doi.org/10.1016/j.cellsig.2023.110775.