Assessment of the immuno-expression profile of breast ductal carcinoma with Cyclin D1, Her-2/Neu and p53 at Yangon General Hospital, Myanmar

Khin Darli Tun[1], Min Ko Ko[2], Sudha Arumugam[1], Srikumar Chakravarthi[1], Jaya Vejayan[3]

[1] Department of Pathology, Faculty of Medicine, MAHSA University, Malaysia

[2]Department of Population and Family Health, University of Public Health (Yangon), Myanmar

[3]Faculty of Industrial Sciences & Technology, Universiti Malaysia Pahang, Kuantan, Pahang, Malaysia

Corresponding author: *Dr Khin Darli Tun, Email: khindar@mahsa.edu.my*

Abstract

One hundred cases of histologically proven invasive ductal carcinomas were histologically graded based on modified Bloom and Richardson Grading. Out of these 100 cases, each of low grade, intermediate grade, and high grade invasive ductal carcinomas were selected for Immunostaining using the monoclonal antibodies Cyclin D1, p53 and Her2/neu. It was found that for all three monoclonal antibodies the lowest histological grade of Invasive Ductal Carcinoma of the Breast showed the lowest positivity with Cyclin D1 (11.76%) and p53 (17.64%) and Her2/neu (47.05%). The intermediate grade tumour showed (70.58%) positivity with Cyclin D1 and 58.58 % in p53 and Her2/neu. The high grade invasive ductal carcinoma of the breast showed the highest positivity of Cyclin D1 (76 .47%), p53 (88.24%), Her2/neu (94.12%); These suggest that Cyclin D1, P53 and Her2/neu immuno-expression positivity increases with rising histological grades of invasive ductal carcinoma of breast.

Key Words: invasive ductal carcinoma, breast, Cyclin D1, p53, Her2/neu

1. Introduction

Breast cancer is the most common cancer affecting females. Cell cycle mediators in breast carcinogenesis are currently well established. Specifically, deregulation of crucial genes that control cell cycle checkpoints has been noted in various breast carcinomas (Malumbres and Barbacid, 2009). Dysfunction or loss of these genes can also mediate resistance to chemotherapeutic agents. Cyclin D1 and its associated cyclin-dependent kinases (CDK4 and CDK6) are central mediators in the transition from G1 to S phase (Roy and Thompson, 2006). In primary breast cancer, it has been shown that the gene encoding cyclin D1 is amplified in

15% of the cases and overexpressed in 30–50% (Zhang, Sakamoto and Wagner, 2014). Of note, elevated levels of cyclin D1 protein have been associated with poor prognosis, whilst overexpression of cyclin D1 has been more commonly found in hormone receptor (HR) positive breast cancer cases (Zhang, Sakamoto and Wagner, 2014). There are aspects in this protein network, in various breast cancer subtypes, that have not been fully understood. New data on the field are more than warranted taking into consideration the introduction of the second generation of highly specific cyclin D1/CDK4/CDK6 inhibitors, agents highly active in metastatic breast cancer (Migliaccio, Di Leo and Malorni, 2014). To the best of our knowledge, very few studies trying to evaluate the prognostic role of elaborate molecular clusters encompassing cyclin D1, p53, Her2/neu in the context of breast cancer among patients in Myanmar.

HER2 overexpression is a frequent event in several human cancers, besides breast tumors, and has been correlated with a poor prognosis (Walker et al, (1991). HER2 is a member of the epidermal growth factor receptor-related family of receptor tyrosine kinases, which comprises HER1, HER2, HER3, and HER4. In breast cancer cells, the mechanism of growth inhibition and differentiation via HER2 receptor activation has been suggested to involve p53, and HER2 overexpression in breast cancer, associated with increased proliferation, is frequently found in tumors with p53 alterations (Dumay et al, 2013).

1.1.Objective

This study aims to determine the immunochemical profile of invasive ductal carcinomas of the breast and its relation to Cyclin D1, Her-2/Neu and p53 immuno-expression at Yangon General Hospital (YGH), Myanmar.

1.2.Materials and Methods

This study is a hospital and laboratory based study, and was done with prior ethical approval and patient consent. A total of 100 pathology specimens of invasive ductal carcinoma were collected and graded according to the Modified Bloom and Richardson Grading Method. All excisional biopsies and mastectomy specimens received from YGH, Histopathology section and Department of Pathology, University of Medicine (I) were collected by the investigators. Description and gross cutting were done meticulously, taking adequate sections from appropriate sites according to the procedure. The inclusion criteria for collected specimens were: All lumpectomy and mastectomy specimens, all recurrent invasive ductal carcinoma of breast. The biopsy specimens were fixed and transported in 10% formalin. The lumpectomy

and mastectomy specimens were sliced in order to achieve adequate fixation. All the specimens were studied grossly and properly labeled. Cyclin D1 and P53 immuno-expression were seen as brown staining on the nucleus of tumour cells. Her 2/neu immuno-expression was seen as brown staining of tumour cells membrane.

A total of histologically proven 51 cases of invasive ductal carcinoma of the breast were further studied. The results from each patient were recorded in the proforma including age, site of the affected area, types of operation, sites of invasive ductal carcinoma, histological grading, Cyclin D1, Her-2/Neu and P53 immuno-expression. All data were checked and cleaned to check outliers, missing data. Analysis was done by Statistical Package for Social Science Study (SPSS) version 11.0 and Microsoft Excel. The categorical variables were summarized as frequency tables. The bivariate analysis with chi-square test was also performed to determine the significance of monoclonal antibodies Cyclin D1, P53, Her2/neu on histological grading of invasive ductal carcinoma of the breast. The p value less than 0.05 set as statistically significant at 95% confident interval.

2. Results

A total of 100 patient samples were chosen for this study. The results were categorized into: Clinical profiles of respondents with Invasive Ductal Carcinoma of the Breast (as shown in Table 1); Immuno-expression in low grade (n= 17), intermediate (n= 17) and high grade (n= 17) invasive ductal carcinoma of breast (as shown in figure 1; and association between grading of Invasive duct Carcinoma Breasts and Immuno-expression (as shown in table 2).

Out of 17 cases of low-grade invasive ductal carcinoma of breast, 4 cases showed negative immuno-expression for Cyclin D1, P53 and Her2/neu (23.63%). The percentage of Cyclin D1 only was positive in 2 cases (11.76%), p53 immuno-expression was positive in 3 cases (17.69%) and Her2/neu

Immuno-expression was positive in 7 cases (47.05%). Out of 17 cases of Intermediate grade invasive ductal carcinoma of the breast 3 cases showed positive immuno-expression for Cyclin D1, P53 and Her2/neu (17.64%). Both Cyclin D1 and P53 were positive in 5 cases (29. 41%). Cyclin D1 and Her2/neu were both positive in 3 cases (17.64%). p53 and Her2/neu immuno-expression were positive in 2 cases (11.74%) Only. Her2/neu positive was seen in 2 cases (11.74%). For all the immuno-histological markers, 2 cases showed negative immuno-expression of Cyclin D1, P53 and Her2/neu. Out of 17 cases of high grade invasive ductal carcinoma all these immuno-histological markers Cyclin D1, P53 and Her2/neu Immuno-

expression were positive in 12 cases, 1 case showed positive for Cyclin D1 and p53, and 4 cases were positive for P53 and Her2/neu, Cyclin D1, P53 and Her2/neu Immuno-expression were negative in 2 cases respectively.

Table 1: Clinical profiles of respondents with Invasive Ductal Carcinoma of the Breast (n= 100)

Background characteristics	Frequency	Percentage
Age group (Years)		
30-39	16	16.0
40-49	26	26.0
50-59	36	36.0
60-69	16	16.0
70-79	4	4.0
80-80	2	2.0
Site		
Left	59	59.0
Right	41	41.0
Types		
Lumpectomy	34	34.0
Mastectomy	66	66.0
Sites of invasive ductal carcinoma		
Left Upper Outer Quadrant	36	36.0
Left Upper Inner Quadrant	9	9.0
Left Lower Outer Quadrant	8	8.0
Left Lower Inner Quadrant	6	6.0
Right Upper Outer Quadrant	25	25.0
Right Upper Inner Quadrant	6	6.0
Right Lower Outer Quadrant	2	2.0
Right Lower Inner Quadrant	8	8.0
Histological Grading		
Low grade	13	13.0
Intermediate grade	36	36.0
High grade	31	31.0

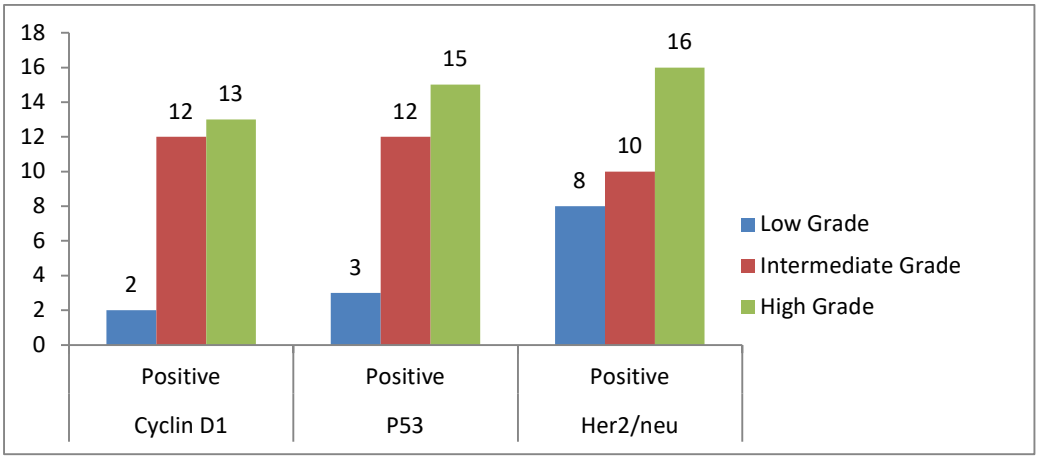

Figure 1: Immuno-expression in low grade (n= 17), intermediate (n= 17) and high grade (n= 17) invasive ductal carcinoma of breast

Table 2: Association between grading of Invasive duct Carcinoma Breasts and Immuno-expressions

Immuno-expressions	Grading			Chi-square	p-value
	Low	Intermediate	High		
Cyclin D1					
Positive	2 (11.8)	12 (70.6)	13 (76.4)	17.47	<0.001
Negative	15 (88.2)	5 (29.4)	4 (23.6)		
P53					
Positive	3 (17.6)	12 (70.6)	15 (88.2)	18.94	<0.001
Negative	14 (82.4)	5 (29.4)	2 (11.8)		
Her 2 neu					
Positive	8 (47.0)	10 (58.8)	16 (94.1)	9.18	0.010
Negative	9 (53.0)	7 (41.2)	1 (5.9)		

3. Discussion

In this study, surgical breast biopsies from all surgical units of YGH and histologically diagnosed as invasive ductal carcinoma of 100 cases were histologically graded by Bloom and Richardson classification, and immunohistochemical profile with CyclinD1, p53 and Her2/neu were studied. Our study involved a total of 100 cases of histologically diagnosed as invasive ductal carcinoma of breast were studied. Out of these cases 17 cases, of low grade, 17 cases of intermediate grade, and 17 cases of high grade invasive ductal carcinomas were selected for Immunohistochemistry stain using the monoclonal antibodies Cyclin D1, p53, Her2/neu. There is an ever-increasing number of markers that can be of value but it is important to ensure that they have been validated in published studies. The majority of these markers are antibodies that

are suitable for use with formalin-fixed, paraffin-embedded (FFPE) tissue. There are many tumor markers that are frequently used in breast cancer, such as myoepithelial markers, Smooth muscle actin, Cytokeratin 14, P-cadherin*, caldesmon, proliferative marker (Ki-67}, protooncogenes, Cyclin D1, p53 and hormonal receptor status using Estrogen receptor, Progesterone receptor, Basement membrane – collagen IV marker, Cytokeratin 5/6, E-cadherin, Epithelial membrane antigen.

In our study the clinical significance of Cyclin D1, P53 and Her2/neu in Invasive ductal carcinoma of Breast was evaluated by peroxidase-anti peroxidase (PAP) method. Immuno-peroxidase methods permit the specific demonstration of cell and tissue antigens in a variety of fixed tissues. For this reason, immune-peroxidase methods have found extensive application in a number of diagnostic and investigative laboratories. The PAP technique is a variation of the basic immune-peroxidase methodology, with the great advantage of a high degree of sensitivity that facilitates the demonstration of antigens in fixed paraffin-embedded tissues. *p53* protein is the product of a tumor suppressor gene located on the human chromosome 17, thought to regulate the proliferation of normal cells. Mutations of *p53* gene have been reported in human breast carcinoma, especially in more advanced and/or more aggressive tumors. (Molina et al, (1998). recently observed *p53* positivity in 37.3% of 655 human breast carcinomas. These authors reported no significant correlation between *p53* expression and tumor size, nodal involvement, or histologic type. However, in another study, (Falette et al, 1998). found that tumors carrying *p53* alterations had a highly aggressive behavior and that the presence of altered *p53* was an independent prognostic marker of early relapse and death. Abnormal *p53* can be used as an independent prognostic indicator of shortened survival and recurrence. Interestingly, it has been postulated that *p53* mutations in codons that directly contact DNA are those able to predict poor outcomes. (Berns et al, 1998). Our study of the immuno-expression of P53 in Low grade invasive ductal carcinoma showed low results with only 3 cases showing the positive result (17.69%) and negative result in 14 cases (82.31%). In intermediate grade, invasive ductal carcinoma P53 positivity was increased up to 12 cases (70.58%) and negative cases were decreased to 5 cases (29.42%). In high grade, invasive ductal carcinoma of the breast the positivity of P53 increased up to 15 cases (88.24%) and negative results were decreased up to 2 cases (11.78%). Therefore, in total 51 cases of invasive ductal carcinoma the P value of .00007702 showed significant results of increasing positivity of p53 with higher histological comparison results with other studies. Although both HER2 overexpression and *p53* mutations

are important prognostic factors for breast cancer, only a few reports have described the signaling cascades that link HER2 and p53 (Taneja et al, 2010).

3.1.Cyclin D1 in breast cancer

Cyclin D1 protein plays an important part in regulating the progress of the cell during the G1 phase of the cell cycle. The cyclin D1 gene, CCND1, is amplified in approximately 20% of mammary carcinomas, and the protein is over-expressed in approximately 50% of cases. This has led to an intensive study to ascertain whether cyclin D1 is a biological marker in breast cancer. In this study, the immuno-expression of Cyclin D1 in Low grade invasive ductal carcinoma showed low results of only 2 cases positivity (11.76%) and negative results in 15 cases (88.24%). In intermediate grade, invasive ductal carcinoma Cyclin D1 positivity increased up to 13 cases (70.58 %) and negative was decreased to 5 cases (29.42%). In high grade invasive ductal carcinoma of breast, the positivity of Cyclin D1 was increased up to 13 cases (76.47%) and negative results were decreased up to 4 cases (23.53%). Therefore, in total 51 cases of invasive ductal carcinoma the P value of .000168068 showed signs of increasing positivity of Cyclin D1 with higher histological grades.

The majority of tumors were positive to cyclin D1 (78.1%) (Zagouri et al, 2017). Cyclin D1 expression was seen in 67.5% of the cases in India study (Ravikumar and Ananthamurthy, 2014). In our study, the Cyclin D1 was associated with tumor grading and more positive Cyclin D1 was found in high-grade malignancy. On one hand, the Cyclin D1 was not associated with the tumor grading of total of 39 consecutive cases of female patients in India (Ravikumar and Ananthamurthy, 2014).

3.2.Her2/neu

The prognostic significance of HER2 over-expression was first reported in 1987. Subsequently, over 200 studies have been reported in which the role of amplification/over-expression of HER2 was investigated as a prognostic marker in breast cancer. Also, we previously reported that HER2 amplification was strongly associated with both disease-free and overall survival in breast cancer show that HER2 over-expression to more than 500 invasive ductal tumors, and showed that over-expression of HER2 was associated with poor prognosis (Yamashita et al, 1993). Nearly one-third of breast cancers have mutations in the p53 gene, which are associated with high histological grade and clinical aggressiveness Immunohistochemical assays generally detect nuclear accumulation of the protein, which is often related to conformational alterations and a prolonged half-life of the encoded protein Accumulation of p53 protein was significantly

associated with poor prognosis in our study and in other studies of patients with breast cancer. These studies suggest both a prognostic and a predictive role for p53 (Allred et al, 1998). Tumors with both HER2 over-expression and p53 protein accumulation were reported in several studies. In this study, the immuno-expression of Her2/neu in 17 cases of Low grade invasive ductal carcinoma showed low positive results in 8 cases (47.05%) and negative results in 9 cases (52.95%). In intermediate grade invasive ductal carcinoma Her2/neu positivity was increased up to 10 cases (58.83%) and negative results decreased to 7 cases (41.17%). In high grade invasive ductal carcinoma of the breast the positivity of Her2/neu was markedly increased up to 16 cases (94.12%) and negative results were decreased up to 1 case (5.88 %). Therefore, in a total 51 cases of invasive ductal carcinoma the P value of <0.05 showed significant results of increasing positivity of Her2/neu with higher histological grading compare with other studies. Her/2neu positivity in our study showed a significant association with tumor grade, which is similar to previous India study (Bansal et al, 2017). In all the 51 cases of Invasive Ductal Carcinoma of the Breast the immuno-expression of Cyclin D1, p53 and Her2/neu increased with increasing histological grades of the tumour. However, In the previous studies, tumor markers are associated with the aggressive disease with poor clinical outcome using survival analysis (Hwangbo et al, 2013; Taneja at al, 2010). A limitation of this study is the survival analysis was not performed and it is called for further analysis. There is evidence that women whose tumours over-express HER2 are likely to derive greater benefit from therapy with anthracycline-containing regimens than from alkylating agents. It was also reported that patients with both HER2 and p53 positive tumors had improved 10-year survival when treated with a high dose FAC regimen. The patients included in the present study were treated with tamoxifen, fluorouracil, or a CMF regimen (fluorouracil, doxorubicin, cyclophosphamide).

4. Conclusion

The prevalence of breast cancer at Yangon General hospital was 31.22%. Out of these 96.1% were ductal carcinoma and 3.9% were lobular carcinoma One hundred cases of histologically proven invasive ductal carcinoma were then histologically graded according to Modified Bloom and Richardson Grading. Out of these 17 cases of low grade, 17 cases of intermediate grade, and 17 cases of high grade invasive ductal carcinomas were selected for Immunostaining using the monoclonal antibodies Cyclin D1, p53 and Her2/neu It was found that for all three monoclonal antibodies the lowest histological grade of Invasive Ductal Carcinoma of the Breast showed the lowest positivity with Cyclin D1 (11.76%) and p53 (17.64%) and Her2/neu (47.05%). The intermediate grade tumour showed 70.58% positivity with Cyclin D1 and

58.58% in p53 and Her2/neu. The high grade invasive ductal carcinoma of the breast showed the highest positivity of Cyclin D1 (76 .47%), p53 (88.24%), Her2/neu (94.12%); These findings suggest that Cyclin D1, p53 and Her2/neu immuno-expression were increased with rising histological grades of invasive ductal carcinoma of breast, in the hospital setting in Myanmar.

Acknowledgment

My deepest gratitude and appreciation to my Professor Dr. Khine San Yin, Professor and Head of the Pathology Department of University of Medicine (I) and Yangon General Hospital for her constructive, continuous guidance, support and advice and for allowing me to use the Histopathology and Immunohistochemistry services at Pathology Department of Yangon General Hospital. My thanks are also due to Dr. Myat Wunna Soe, Public Health Specialist, Director General of Medical Services, Ministry of Health, for kind help with the statistical analysis of the data obtained. Finally, I would like to thank all the staff of the Histopathology Section, Yangon General Hospital and staff of the Library of University of Medicine (I) for their technical help and library services provided for literature search.

References

Allred, D. C., Harvey, J. M., Berardo, M., & Clark, G. M. (1998). Prognostic and predictive factors in breast cancer by immunohistochemical analysis. Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc, 11(2), 155–168.

Bansal, C., Sharma, A., Pujani, M., Pujani, M., Sharma, K. L., Srivastava, A. N., & Singh, U. S. (2017). Correlation of Hormone Receptor and Human Epidermal Growth Factor Receptor-2/neu Expression in Breast Cancer with Various Clinicopathologic Factors. Indian journal of medical and paediatric oncology: official journal of Indian Society of Medical & Paediatric Oncology, 38(4), 483–489. (online). Available at: https://doi.org/10.4103/ijmpo.ijmpo_98_16

Berns, E. M., van Staveren, I. L., Look, M. P., Smid, M., Klijn, J. G., & Foekens, J. A. (1998). Mutations in residues of TP53 that directly contact DNA predict poor outcome in human primary breast cancer. British journal of cancer, 77(7), 1130–1136. (online). Available at: https://doi.org/10.1038/bjc.1998.187

Dumay, A., Feugeas, J. P., Wittmer, E., Lehmann-Che, J., Bertheau, P., Espié, M., Plassa, L. F., Cottu, P., Marty, M., André, F., Sotiriou, C., Pusztai, L., & de Thé, H. (2013). Distinct tumor protein p53 mutants in breast cancer subgroups. International journal of cancer, 132(5), 1227–1231. (online). Available at: https://doi.org/10.1002/ijc.27767

Falette, N., Paperin, M. P., Treilleux, I., Gratadour, A. C., Peloux, N., Mignotte, H., Tooke, N., Löfman, E., Inganäs, M., Bremond, A., Ozturk, M., & Puisieux, A. (1998). Prognostic value of P53 gene mutations in a large series of node-negative breast cancer patients. Cancer research, 58(7), 1451–1455.

Hwangbo, W., Lee, J. H., Ahn, S., Kim, S., Park, K. H., Kim, C. H., & Kim, I. (2013). EGFR Gene Amplification and Protein Expression in Invasive Ductal Carcinoma of the Breast. Korean journal of pathology, 47(2), 107–115. (online). Available at: https://doi.org/10.4132/KoreanJPathol.2013.47.2.107

Malumbres M, Barbacid M. (2009) Cell cycle, CDKs and cancer: a changing paradigm, Nat Rev Cancer, 9(3):153–66. (online). Available at: https://doi.org/10.1038/nrc2602, PMID: 19238148.

Migliaccio, I., Di Leo, A., & Malorni, L. (2014). Cyclin-dependent kinase 4/6 inhibitors in breast cancer therapy. Current opinion in oncology, 26(6), 568–575. (online). Available at: https://doi.org/10.1097/CCO.0000000000000129

Molina, R., Segui, M. A., Climent, M. A., Bellmunt, J., Albanelll, J., Fernandez, M., Filella, X., Jo, J., Gimenez, N., Iglesias, E., Miralles, M., Alonso, C., Peiro, G., Perez-Picañol, E., & Ballesta, A. M. (1998). p53 oncoprotein as a prognostic indicator in patients with breast cancer. Anticancer research, 18(1B), 507–511.

Ravikumar, G., & Ananthamurthy, A. (2014). Cyclin D1 expression in ductal carcinoma of the breast and its correlation with other prognostic parameters. Journal of cancer research and therapeutics, 10(3), 671–675. (online). Available at: https://doi.org/10.4103/0973-1482.138135

Roy, P. G., & Thompson, A. M. (2006). Cyclin D1 and breast cancer. Breast (Edinburgh, Scotland), 15(6), 718–727. (online). Available at: https://doi.org/10.1016/j.breast.2006.02.005

Taneja, P., Maglic, D., Kai, F., Zhu, S., Kendig, R. D., Fry, E. A., & Inoue, K. (2010). Classical and Novel Prognostic Markers for Breast Cancer and their Clinical Significance. Clinical Medicine Insights. Oncology, 4, 15–34. (online). Available at: https://doi.org/10.4137/cmo.s4773

Walker, R. A., Dearing, S. J., Lane, D. P., & Varley, J. M. (1991). Expression of p53 protein in infiltrating and in-situ breast carcinomas. The Journal of pathology, 165(3), 203–211. (online). Available at: https://doi.org/10.1002/path.1711650303

Yamashita, H., Kobayashi, S., Iwase, H., Itoh, Y., Kuzushima, T., Iwata, H., Itoh, K., Naito, A., Yamashita, T., & Masaoka, A. (1993). Analysis of oncogenes and tumor suppressor genes in human breast cancer. Japanese journal of cancer research: Gann, 84(8), 871–878. (online). Available at: https://doi.org/10.1111/j.1349-7006.1993.tb02060.x

Zagouri, F., Kotoula, V., Kouvatseas, G., Sotiropoulou, M., Koletsa, T., Gavressea, T., Valavanis, C., Trihia, H., Bobos, M., Lazaridis, G., Koutras, A., Pentheroudakis, G., Skarlos, P., Bafaloukos, D., Arnogiannaki, N., Chrisafi, S., Christodoulou, C., Papakostas, P., Aravantinos, G., Kosmidis, P., … Fountzilas, G. (2017). Protein expression patterns of cell cycle regulators in operable breast cancer. PloS one, 12(8), e0180489. (online). Available at: https://doi.org/10.1371/journal.pone.0180489

Zhang, Q., Sakamoto, K., & Wagner, K. U. (2014). D-type Cyclins are important downstream effectors of cytokine signaling that regulate the proliferation of normal and neoplastic mammary epithelial cells. Molecular and cellular endocrinology, 382(1), 583–592. (online). Available at: https://doi.org/10.1016/j.mce.2013.03.016