ANTICANCER RESEARCH *36*: xxx-xxx (2016)

No: 5622-C
Please mark the appropriate section for this paper
☐ Experimental
☐ Clinical
☐ Epidemiological

Neural Analyses Validate and Emphasise the Role of Progesterone Receptor in Breast Cancer Progression and Prognosis

ARTURO CARONONGAN III[1], BARBARA VENTURINI[2], DEBORA CANUTI[3], SATNAM DLAY[4], RAOUF N.G. NAGUIB[5,6] and GAJANAN V. SHERBET[4,7]

[1]*College of Computer Studies, De La Salle University, Manila, Philippines;*
[2]*Oncology Department, and* [3]*Cancer Screening Centre, Romagna Local Health Unit, Rimini, Italy;*
[4]*School of Electrical and Electronic Engineering, University of Newcastle upon Tyne, U.K.;*
[5]*BIOCORE Research & Consultancy International, Liverpool, U.K.;*
[6]*Faculty of Science, Liverpool Hope University, Liverpool, U.K.;*
[7]*Institute for Molecular Medicine, Huntington Beach, CA, U.S.A.*

Abstract. *Oestrogen receptor (ER) expression is routinely measured in breast cancer management, but the clinical merits of measuring progesterone receptor (PR) expression have remained controversial. Hence the major objective here was to assess the potential of PR as a predictor of response to endocrine therapy. We report analyses of the relative importance of ER and PR for predicting prognosis using robust multilayer perceptron artificial neural networks. Receptor determinations use immunohistochemical (IHC) methods or radioactive ligand binding assays (LBA). In view of the heterogeneity of intratumoral receptor distribution, we examined the relative merits of the IHC and LBA methods. Our analyses reveal a more significant correlation of IHC-determined PR than ER with both nodal status and 5-year disease-free survival (DFS). In LBA, PR displayed higher correlation with survival and ER with nodal status. There was concordance of correlation of PR with DFS by both IHC and LBA. This study suggests a clear distinction between PR and ER, with PR displaying greater correlation than ER with disease progression and prognosis, and emphasises the marked superiority of the IHC method over LBA. These findings may be valuable in the management of patients with breast cancer.*

Breast cancer treatment and patient management is based on the state of progression of the disease. The assessment of prognosis and determination of the mode of treatment has traditionally relied on histological grade, tumour stage, vascular and lymphatic invasion and hormone receptor status. Various combinations of these are employed to predict prognosis and determine risk groups. But the utility of this approach might be limited since it cannot be employed in determining the prognosis of individual patients. In recent years, much effort has been made to identify molecular and cell markers that might have the potential to assess the state of progression and predict prognosis accurately. Molecular subtyping of breast cancers into luminal A [oestrogen receptor (ER) and/or progesterone receptor (PR^+)/epidermal growth factor receptor ($HER2)^-$; low Ki67)], luminal B [ER^+ and/or $PR^+/HER2^+$ (or $HER2^-$ with high Ki67)], HER2 subtype ($HER2^+/ER^-/PR^-$) and basal-like ($ER^-/PR^-/HER2^-$/ cytokeratin $5/6^+$ or $EGFR^+$) (1) reflects tumour aggressiveness and prognosis. It is often applied in research since individually these parameters are known to influence tumour growth, dissemination and progression. However, the complexity associated with the subtyping system and the panel of parameters involved has made extracting clinically valuable prognostic information somewhat difficult. Molecular subtyping implicitly embraces the relative importance of ER and PR as prognostic markers.

Historically, ER and PR are known to affect the growth of a variety of tissues, including breast tissue. ER^- tumours are resistant to anti-oestrogen therapy, display rapid growth and result in poor outcome for patients. That PR expression might be an important prognostic marker was suggested some years ago (2, 3). However, the measurement of PR expression in patient management has not been accorded a significant role and more emphasis on assessing PR status has been advocated (4), Functional PR might be required for proper growth signalling by ER. Patients with breast cancer

Correspondence to: Gajanan V. Sherbet, School of Electrical and Electronic Engineering, Merz Court, University of Newcastle upon Tyne NE1 7RU, U.K. E-mail: gajanan.sherbet@ncl.ac.uk

Key Words: Breast cancer, multilayer perceptron artificial neural networks, oestrogen receptor, progesterone receptor, progression, prognosis.

expressing both receptors have the best prognosis and are more likely to respond to hormone treatment than patients with ER^-/PR^- tumours (5). Combining ER/PR expression with characteristics of cell proliferation can accurately predict nodal involvement and 5-year disease-free survival (DFS) of patients with breast cancer (6). Patients with PR^+ disease appear to respond better to hormonal therapies and survive longer (7). Dowsett *et al*. found that patients with breast cancer with low ER, PR and HER2 treated with tamoxifen and the aromatase inhibitor anastrozole had poor prognosis and recurrence was also inversely related to receptor expression (8). Recently, Purdie *et al*. reported that the molecular subtype luminal A reflects the best prognosis (9). Indeed, patients with PR+ disease have not only been known to respond to endocrine therapy but have also been regarded to have good prognosis in terms of overall survival (OS). The importance of PR is underscored by the suggestion that the luminal A subtype should be redefined on account of the consistently higher expression of PR in that subtype as compared with luminal B (10). Several years ago, Horwitz and McGuire described PR as an oestrogen-responsive gene (2). But some ER^-/PR^+ tumours display greater response to endocrine therapy than ER^-/PR^- tumours, suggesting that PR may lead to good clinical outcome independently of ER (5, 11). Finally, it ought to be recalled here that three PR isoforms, namely PRA, PRB and PRC, have been identified, which appear to have different functions (12). The differential roles of PR isoforms was emphasised some time ago, with high PRA expression being correlated with tumour relapse, and breast cancer 1 (*BRCA1*) and breast cancer 2 (*BRCA2*) mutations being associated with high PRA expression (13, 14). Differential expression of the isoforms has been linked with methylation of the PR gene and is associated with outcome (15).

Since ER can induce the expression of PR, a reduced response to hormones in patients with ER+ disease could indicate a non-functional state of ER. In breast cancer cells, both oestrogen and progesterone can activate the SRC/extracellular signal – regulated kinase (SRC/ERK) pathway and promote cell proliferation. Ballaré *et al*. have attributed this to the presence of two domains of PR which interact with ER (16). Conversely, PR has now been recognised to be able to regulate ER function in breast cancer (17). Progesterone was also shown to negatively regulate other oestrogen-regulated signalling pathways, leading to the inhibition of proliferation (18). The importance of ER/PR is further highlighted by the possibility that ER/PR signalling can interact with the p53 pathway. Mouse double minute 2 homolog (MDM2) is known to mediate the function of both $p14^{ARF}$ and p53. It is of interest to note in this context that ER has been implicated in the regulation of the p14ARF–MDM2–p53 pathway (19). Furthermore, ER has been linked with signalling by epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor 2 (HER2). Both EGFR and the HER2 are said to activate ER and its co-activator amplified in breast cancer-1 (AIB1) (20). Given that both growth factor receptors can collaborate in generating phenotypic effects *via* activation of nuclear factor kappa B/Phosphatidylinositol-3-kinase and protein kinase B (NFκB/PI3K-AKT) activation, ER signalling is rightly credited with a major role in cancer progression (21).

With this background, it seemed eminently worthwhile to attempt to determine the relative, as well as individual, relevance of ER and PR to breast cancer progression. The clinical potential of PR expression in breast cancer is controversial. Hence the major objective of this work was to assess the potential of PR as a predictor of response to endocrine therapy.

Receptor assessments are made on a routine basis using immunohistochemical (IHC) techniques. However IHC is best described as grading tumours for receptor expression. Assays that utilise radioactive ligands (LBA) measure the actual levels of receptor present in a tumour but, unlike IHC, give an overall picture of expression without providing information on the intratumoral distribution of receptors. Intratumoral heterogeneity is not an uncommon feature and heterogeneity in the distribution of many markers including PR, ER, p53 and the proliferative marker Ki-67 (MIB) is encountered in breast cancer (22), so much so that small biopsies might provide an inaccurate picture of receptor status. Therefore the present study set itself another objective *i.e*. comparing the efficiency of IHC and radioactive assays in prediction of progression of the disease and patient survival.

Materials and Methods

Clinical data and methodology. The datasets used in this study include ER and PR determinations using IHC and LBA of breast tumours from 110 patients, together with the state of nodal involvement and 5-year survival data. ER and PR status was determined by conventional methods of IHC and LBA assays (23). The full datasets analysed here are given in Tables I and II.

Methods of analysis. Initial stages of the study looked to determine a close approximation of a patient's survival period given the data provided, thus the Multi-Layer Perceptron (MLP) approach was considered. The MLP is an artificial neural network model that behaves through a feedforward mechanism providing a set of outputs based on the given input data (24). This is the computational model used in previous research which aimed for the prediction of a specific output upon provision of certain parameters as input data. This was the model of choice in this study, since it has been used in several clinical studies to predict risk of mortality of stroke patients and risk of haemorrhage in ischaemic stroke (25, 26) and for diagnosing cardiovascular diseases (27, 28). Essentially, the model involves assigning individual cases to a specified number of classes and determining how many were correctly or incorrectly classified.

Table I. *Oestrogen receptor (ER) and progesterone receptor (PR) levels in breast cancer as assayed by immunohistochemistry.*

Patient ID	ER (+fraction)	PR (+fraction)	Nodal status, % (positive nodes/total nodes tested)	Date of follow-up: Surgery to last seen
196	0.8	0.7	11.1 (3/27)	1997-2003
199	0	0	6.7 (1/15)	1997-2003
200	0.8	0.1	0 (0/14)	1998-2003
201	0	0	0 (0/4)	1999-2003
203	0.4	0.25	25 (4/16)	1999-2003
204	0.9	0	0 (0/26)	1999-2004
205	0	0.2	0 (0/19)	2000-2003
206	0.22	0	7.7 (1/13)	1998-2004
209	0.46	0.08	5.5 (1/18)	1998-2000
213	0	0	0 (0/27)	1999-2003
214	0.1	0.2	12.5 (3/24)	1997-2005
219	0.95	0.16	0 (0/18)	1999-2004
222	0.7	0.2	5 (1/20)	1996-2003
225	0.1	0.4	7.7 (1/13)	1999-2004
226	0.75	0	0 (0/20)	1999-2003
227	0.9	0.33	6.2 (1/16)	1999-2003
238	0.9	0.32	0 (0/22)	1999-2000
231	0	0	0 (0/20)	1998-2003
233	0.9	0.9	0 (0/11)	1997-1998
257	0.5	0	0 (0/16)	1999-2004
280	0	0.9	0 (0/9)	1999-2003
294	0.8	0.15	62.5 (10/16)	1994-2001
297	0.5	0.3	0 (0/25)	1994-2003
309	0.9	0.1	30 (3/10)	1999-2003
238	0	0	0 (0/12)	2003-2004
240	1.0	1.0	0 (0/20)	2001-2004
241	0.05	0.05	9 (1/11)	2001-2003
243	0.9	0.8	0 (0/3)	2001-2003
245	0	0	0 (0/25)	2002-2004
246	0	0	0 (0/13)	2002-2004
249	1.0	0	4 (1/25)	2004-2004
250	0.6	0	63.6 (14/22)	2002-2004
252	0	0	0 (0/16)	2001-2004
254	0.8	0.49	0 (0/18)	2002-2004
255	0	0.1	0 (0/19)	2003-2003
258	0.8	0.6	0 (0/18)	2003-2004
259	0.6	0.05	0 (0/16)	2002-2003
260	0.9	0.8	31 (5/16)	2002-2003
261	0.8	0.8	0 (0/19)	2001-2003
262	0.9	0.7	0 (0/14)	2001-2004
263	0.9	0.1	14 (2/14)	2004-2004
264	0.8	0.8	33 (4/12)	2001-2004
283	0	0	96 (24/25)	2002-2003
267	0.7	0.9	10 (1/10)	2003-2004
288	0.9	0.9	0 (0/11)	2000-??
311	0	0	43.7 (7/16)	2003-2004
312	0.95	0.95	0 (0/21)	2003-2003
313	0.9	0.4	0 (0/20)	2003-2004
314	0.7	0.7	11 (2/18)	2003-2004
315	0.05	0.05	0 (0/7)	2001-2004
316	0.95	0.95	10 (2/20)	2001-2004
317	0.9	0.7	0 (0/29)	2004-??
318	0	0	3.5 (1/28)	2003-2004
319	0.7	0.95	0 (0/14)	2002-??

Table II. *Oestrogen receptor (ER) and progesterone receptor (PR) levels in breast cancer measured by ligand-binding assay*.*

Patient ID	ER (fmol)	PR (fmol)	Nodal status, % (positive nodes/total) nodes tested	Date of follow-up: Surgery to last seen
183	38	140	0 (0/18)	1996-2003
197	80-500	2	7.1 (1/14)	1997-2003
215	80-484	90-500	0 (0/25)	1997-2001
216	34-60	65	0 (0/12)	1996-2000
218	64	42	100 (14/14)	1996-2000
229	22	11	100 (20/20)	1996-1999
230	55-80	72	5.3 (1/19)	1999-2003
232	34-90	90-500	0 (0/12)	1999-2003
234	90-500	10-130	10 (1/10)	1996-2004
235	3.8	1.1	0 (0/17)	1996-2000
236	0-2.8	0-2.2	5.5 (1/18)	1997-2000
266	3	80	0 (0/12)	1992-2004
267	14	50	21.4 (3/14)	1992-2003
268	5	2	0 (0/15)	1992-2003
269	27	6	0 (0/15)	1992-1999
270	122	63	11 (1/9)	1992-2003
271	1	0	5.5 (1/18)	1992-2003
272	1	4	0 (0/22)	1992-2001
274	1-4	5-1	16.6 (2/12)	1994-1997
275	0-1	0-2	0	1994-2004
276	58	388	20 (3/15)	1994-2003
277	15-17	10-16	60 (9/15)	1994-2004
278	2	8	0 (0/15)	1994-2002
279	0-50	0	5.5 (1/18)	1993-1999
281	212	43-250	0 (0/13)	1993-1997
282	35-50	70-1077	28.5 (4/14)	1993-1996
292	158	24	0 (0/19)	1993-2003
293	161	160	33.3 (4/12)	1993-1998
295	90-755	95-167	14.3 (3/21)	1993-2003
296	18	5	27.3 (6/22)	1992-2003
298	22	34	0 (0/17)	1992-2002
299	93	207	0 (0/32)	1994-2001
300	5	9	0 (0/23)	1993-2002
301	183	8	0 (0/21)	1993-2000
302	15-20	13	0 (0/13)	1993-2003
305	1	4	10 (2/20)	1992-1995
306	222	84	4.5 (1/22)	1993-??
307	550	415	0 (0/16)	1994-2004

*Note: Where a range of values is shown, the lower value was used in the analysis.

In other words, using the MLP technique, predictions are made of the outcome, here in terms of nodal involvement and 5-year DFS, where the correlation coefficients reflect the accuracy of classification. Rank tests are not applied to verify the statistical significance of the observation since there is a general perception that unlike MLPs, they do not adequately meet the requirements for performing multiple comparisons.

The analytical work was conducted using the University of Waikato's WEKA Machine Learning Tool (29). As the sample size was small for the MLP to generate an actual prediction, the next

Table III. *Correlation coefficients of oestrogen receptor (ER) and progesterone receptor (PR) with nodal spread and disease-free survival (DFS) – a comparison between immunohistochemistry (IHC) and ligand-binding assay (LBA)*

	IHC				LBA			
Parameter	Nodal spread	*p*-Value	DFS	*p*-Value	Nodal spread	*p*-Value	DFS	*p*-Value
ER	–0.034	0.81	–0.081	0.571	–0.086	0.614	0.151	0.371
PR	–0.119	0.403	–0.195	0.171	–0.048	0.778	0.154	0.362

Table IV. *Results obtained in relation to oestrogen receptor (ER) and progesterone receptor (PR) ratio.*

Parameter tested	IHC	LBA
Average years of survival	3.05	7.15
Proportion surviving longer than average	69.23%	64.71%
Coefficient of correlation with prognosis	0.66 (p=0.054)	0.77 (p=0.005)

step was designed to determine the possibility of classifying whether a patient would be able to survive longer than the 5-year relative survival rate, as indicated by the National Cancer Institute's Surveillance, Epidemiology, and End Results Program database (30). Adding a new label that is nominal in value produced preliminary results (as discussed in the next section) that involved an output of determining whether a patient's prognosis would be a survival period longer than 5 years, which required ER, PR, and nodal status as input parameters. Despite the preliminary results, further analysis of the dataset involved a large bias towards longer survival, thus there was a need to experiment with other methods.

The next phase of experimentation focused on determining whether PR or ER better correlated with disease progression. The linear association between the continuous values (31) of ER or PR and the patient's prognosis was assessed here. This approach was used on both IHC and LBA datasets to determine whether ER or PR correlated with the patient's prognosis.

Due to the results from the previous phase, further analyses were performed where the ratio of ER to PR was analysed to determine if a relative expression factor presented as a ratio significantly influenced the prediction of prognosis. As the previous phase displayed a low correlation factor, further examination showed that the computation was affected by the varying prognosis of two patients who had the same given values in the ER and PR field. An example of such scenario is patient 231 compared to patient 238 from the IHC dataset indicated in Table I, where both patients had the value of 0 provided for both ER and PR, but the prognosis was 5 years for patient 231 and only 1 year for patient 238. To address this issue, entries of the datasets were grouped into random subgroups within the dataset. Each subgroup was generated randomly and with a threshold of at least 60% of the dataset included in the subgroup, where there were no duplicate entries in any subgroup produced. Subsequently, the correlation approach was applied to each subgroup.

The aforementioned approach was applied to both the IHC and LBA datasets, and each produced a possible moderate and high positive correlation (32) as discussed in the next section.

Results

Our analyses undertaken through the MLP approach suggest that IHC-determined PR expression shows greater correlation than ER with both nodal status and 5-year DFS. But in the LBA measurements, PR displayed higher correlation with survival and ER with nodal status (Table III). The correlation values were not sufficiently robust and weighty; it is nonetheless of interest to note the apparent concordance of correlation of PR with DFS by both IHC and LBA. These conclusions are subject to the caveat that performance rating of IHC and LBA from the same dataset would have been more robust and persuasive. Furthermore, the discriminatory effects, as indicated by the correlation coefficients resulting from the MLP approach, are limited in this study due to the small sample size and the biased values for prognosis which are more heavily skewed towards surviving longer than the 5-year life expectancy.

Further analysis of the datasets indicated a possible correlation between the ratio of ER to PR and the prognosis of a patient's outcome. The results presented in Table IV were obtained by grouping users in the dataset who survived more than the average number of years. Regarding the patients who survived more than the average number of years in the IHC dataset, a correlation coefficient of 0.66 for ER/PR ratio in relation to prognosis was found. This approach was also applied to the LBA dataset and produced a highly significant correlation coefficient of 0.77.

While the reference or orientation is small, the positive correlation of the ER/PR ratio with patient prognosis was present in both the IHC and LBA datasets, indicating the possibility of this being a better indicator for prognosis.

These findings should be further corroborated through the analysis of a larger dataset.

Discussion

The rationale of the analyses. This investigation focused on three areas relevant to the role of steroid receptor determination in the management of patients with breast cancer. The first was to determine whether measurement of PR in breast cancer tissues makes a significant contribution to the prediction of the ability of breast cancer to spread to regional lymph nodes and to the prediction of 5-year DFS of patients. Given the emphasis on nodal involvement, we addressed the early events of secondary spread and not the late recurrences attributable to the presence of micro-metastases at the onset of disease. Consistent with this is the recent finding that low PR expression together with the presence of Ki67 in primary tumour as well as metastases is predictive of the pace of progression of breast cancer (33). Considered co-operatively, the ER^+/PR^+ and ER^+/PR^- phenotypes do not seem to differ much from the ER^-/PR^- phenotype in terms of pathological features and prognosis (34). Indeed, it has been suggested that the benefits to patients of anti-oestrogen therapy might be superior when PR is expressed alone than when co-expressed with ER (35). The second point of focus was the relative importance of ER and PR expression, individually, and in tandem with clinical outcome. Finally, with practical implications in mind, a comparison of the efficacy of the two methods used in the measurement of the receptors, namely IHC and LBA assays, to predict clinical outcome, was carried out.

PR status correlates with nodal spread and DFS. In the present study, far superior correlation of PR with both nodal status and DFS was noted. In contrast, ER determined by LBA correlated only with nodal status. This is intriguing given that LBA determines the overall receptor content irrespective of intratumoral distribution, which could be a major determinant of tumour behaviour. Overall, the present work clearly underscores earlier findings that PR has an important contribution to make in determining the course of patient management.

Comparison of IHC and LBA in relation to clinical outcome. The IHC assay is used to determine the proportion of cells that stain positively with the respective antibodies and tumours which are graded positive or negative using arbitrary cut-off levels of positivity. However, the choice of cut-off values is not standardised; hence in the present study, the percentage of positive cells in each tumour was employed for the analyses.

Notwithstanding the problems associated with the absence of standardisation, IHC assay has been regarded as more appropriate than the LBA. It has been argued that ER assessment by IHC is superior to LBA in that the former is easier and less expensive and indeed might be more efficient for predicting response to adjuvant endocrine therapy (36). Moreover, claims have been made that IHC provides more useful information relating to the correlation of PR and ER with clinical outcome than that obtained using LBA (37, 38).

However, a high degree of concordance of positivity has been observed for both ER and PR determined by the two methods (39, 40).

Our analyses revealed a more significant correlation of IHC-determined PR than ER with both nodal status and 5-year DFS. In LBA, PR displayed higher correlation with survival and ER with nodal status, and these findings might be helpful at the clinical level.

Conclusion

The issues of the development of resistance or determination of sensitivity to endocrine therapy are highly relevant in the context of therapeutic strategy. The evaluation of the relative potential of conventional markers is essential in order to develop new molecular approaches to breast cancer management. This study draws a clear distinction between PR and ER, with PR displaying greater correlation than ER with regard to their relationship with disease progression and prognosis, and emphasises the marked superiority of the IHC method over LBA. These findings may be valuable in the management of patients with breast cancer.

Acknowledgements

GVS thanks Professor Barrie Mecrow for the provision of research facilities.

Conflicts of Interest

GVS confirms on behalf of all the Authors that there are no conflicts of interest in regard to this study..

Ethical Approval

Ethical approval not required.

References

1 Schnitt SJ: Classification and prognosis of invasive breast cancer: from morphology to taxonomy. Modern Pathol *23*: S60-S64, 2010.

2 Horwitz KB and McGuire WL: Predicting response to endocrine therapy in human breast cancer: A hypothesis. Science *189*: 726-727, 1975.

3 Ravdin PM, Green S, Dorr TM, McGuire WL, Fabian C, Carter RD, Rivkin SE, Borst JR, Belt RJ, Metch B and Osborne CK: Prognostic significance of progesterone receptor levels in

estrogen receptor-positive patients with metastatic breast cancer treated with tamoxifen: Results of a prospective Southwest Oncology Group Study. J Clin Oncol *10*: 1284-1291, 1992.

4 Stendahl M, Ryden L, Nordenskjold B, Jonsson PE, Landberg G and Jirstrom K: High progesterone receptor expression correlates to the effect of adjuvant tamoxifen in premenopausal breast cancer patients. Clin Cancer Res *12*: 4614-4618, 2006.

5 Osborne CK: Steroid hormone receptors in breast cancer management. Breast Cancer Res Treat *51(3)*: 227-238, 1998.

6 Andronas M, Dlay SS and Sherbet GV: Oestrogen and progesterone receptor expression influences DNA ploidy and the proliferation potential breast cancer cells. Anticancer Res *23*: 3029-3039, 2003.

7 Lapidus RG, Nass SJ and Davidson NE: The loss of estrogen and progesterone receptor gene expression in human breast cancer. J Mammary Gland Biol Neoplasia *3*: 85-94, 1998.

8 Dowsett M, Allred C, Knox J, Quinn E, Slater J, Wale C, Cuzick J, Houghton J, Williams N. Mallon E. Bishop H, Ellis I, Larsimont D, Sasano H, Carder P, Cussac AL. Knox F, Speirs V, Forbes J and Buzdar A: Relationship between quantitative estrogen and progesterone receptor expression and human epidermal growth factor receptor 2 (HER-2) status with recurrence in the arimidex, tamoxifen, alone or in combination trial. J Clin Oncol *26*: 1059-1065, 2008.

9 Purdie CA, Quinlan P, Jordan LB, Ashfield A, Ogston S, Dewar JA and Thompson AM: Progesterone receptor expression is an independent prognostic variable in early breast cancer: A population-based study. Br J Cancer *110*: 565-572, 2014.

10 Prat A, Chang MC, Martin M, Parker JS, Carrasco E, Caballero R, Tyldesley S, Gelmon K, Bernard PS, Nielsen TO and Perou CM: Prognostic significance of progesterone receptor-positive tumor cells within immunohistochemically defined luminal A breast cancer. J Clin Oncol *31*: 203-309, 2013.

11 Osborne CK, Schiff R, Arpino G, Lee AS and Hilsenbeck VG: Endocrine responsiveness: Understanding how progesterone receptor can be used to select endocrine therapy. Breast *14*: 458-465, 2005.

12 Kariagina A, Aupperlee MD and Haslam SZ: Progesterone receptor isoform functions in normal breast development and breast cancer. Crit Rev Eukaryol Gene Expr *18*: 11-33, 2008.

13 Hopp TA, Weiss HL, Hilsenbeck SG, Cui YK, Allred DC, Horwitz KB and Fuqua SAW: Breast cancer patients with progesterone receptor PR-A-rich tumors have poorer disease-free survival rates. Clin Cancer Res *10*: 2751-2760, 2004.

14 Mote PA, Leary JA, Avery KA, Sandelin K, Chenevix-Trench G, Kirk JA and Clarke CL: Germ-line mutations in *BRCA1* or *BRCA2* in the normal breastare associated with altered expression of estrogen-responsive proteins and the predominance of progesterone receptor A. Genes Chrom Cancer *39*: 236-248, 2004.

15 Pathiraja TN, Shettty PR, Jlinek J, He R, Hartmaier R, Margossian AL, Hilsenbeck SG, Issa JPJ and Oesterreich S: Progesterone receptor isoform-specific promoter methylation – association of PRA promoter methylation with worse outcome in breast cancer patients. Clin Cancer Res *17*: 4177-4186, 2011.

16 Ballaré C, Uhrig M, Bechtold T, Sancho E, Di Domenico M, Migliaccio A, Auricchio F and Beato M: Two domains of the progesterone receptor Interact with the estrogen receptor and are required for progesterone activation of the c-SRC/ERK pathway in mammalian cells. Mol Cell Biol *23*: 1994-2008, 2003.

17 Mohammed H, Russell IA, Stark R, Rueda OM, Hickey TE, Tarulli GA, Serandour AAA, Birrell SN, Bruna A, Saadi A, Menon S, Hadfield J, Pugh M, Raj GV, Brown GD, D'Santos C, Robinson JLL, Silva G, Launchbury R, Perou CM, Stingl J, Caldas C, Tilley WD and Carroll JS: Progesterone receptor modulates ER alpha action in breast cancer. Nature *523*: 313-317, 2015.

18 Chen B, Pan H, Zhu L, Deng Y and Pollard JW: Progesterone inhibits the estrogen-induced phosphoinositide 3-kinase–>AKT–>GSK-3beta–>cyclin D1–>pRB pathway to block uterine epithelial cell proliferation. Mol Endocrinol *19*: 1978-1990, 2005.

19 Cho EY, Choi YL, Chae SW, Sohn JH and Ahn GH: Relationship between p53-associated proteins and estrogen receptor status in ovarian serous neoplasms. Int J Gynecol Cancer *16*: 1000-1006, 2006.

20 Osborne CK and Schiff R: Growth factor receptor cross-talk with estrogen receptor as a mechanism for tamoxifen resistance in breast cancer. Breast *12*: 362–367, 2003.

21 Sherbet GV: Growth Factors and Their Receptors in Cell Differentiation, Cancer and Cancer Therapy. Elsevier, London, UK, 2011.

22 Nassar A, Radhakrishnan A, Cabrero IA, Cotsonis GA and Cohen C: Intratumoral heterogeneity of immunohistochemical marker expression in breast carcinoma: A tissue microarray-based study. Appl Immunohistochem Mol Morphol *18*: 433-441, 2010.

23 Mojarad S, Venturini B, Fulgenzi F, Papaleo R, Brisigotti M, Monti F, Canuti D, Ravaioli A, Woo L, Dlay S and Sherbet GV: Prediction of nodal metastasis and prognosis of breast cancer by ANN-based assessment of tumour size and p53, Ki-67 and steroid receptor expression. Anticancer Res *33*: 3925-3933, 2013.

24 Haykin S: Neural Networks: A Comprehensive Foundation. Second Edition.,Prentice Hall, Upper Saddle River, New Jersey, USA, 1998.

25 Süt N and Çelik Y: Prediction of mortality in stroke patients using multilayer perceptron neural networks. Turkish J Med Sci *42*: 886-893, 2012.

26 Bentley P, Ganesalingam J, Jones ALC, Mahady K, Epton S, Rinne P, Sharma P, Halse O, Mehta A and Rueckert D: Prediction of stroke thrombolysis outcome using CT brain machine learning. NeuroImage: Clinical *4*: 635-640, 2014.

27 Yan H, Jiang Y,Zheng J, Peng C and Li Q: A multilayer perceptron-based medical decision support system for heart disease diagnosis. Expert Sys Apps *30*: 272-281, 2006.

28 Panday SP and Godara N: Decision support system for cardiovascular heart disease diagnosis using improved multilayer perceptron. Int J of Computer Appl *45*: 12-20, 2012.

29 Hall M, Frank E, Holmes G, Pfahringer B, Reutemann P and Witten I: The WEKA data mining software: An update. SIGKDD Explorations *11*: 10-18, 2009.

30 SEER Stat Fact Sheets: Breast Cancer, 2012. Online, available from: <http://seer.cancer.gov/statfacts/html/breast.html>, Accessed May 17, 2015.

31 Mukaka M: A guide to appropriate use of correlation coefficient in medical research. Malawi Med J *24*: 69-71, 2012.

32 Hinkle DE, Wiersma W and Jurs SG: Applied Statistics for the Behavioral Sciences. Fifth Edition. Boston: Houghton Mifflin, 2003.

33 Rocca A, Farolfi A, Maltoni R, Carretta E, Melegari E, Ferrario C, Cecconetto L, Sarti S, Schirone A, Fedeli A, Andreis D, Pietri E, Ibrahim T, Montalto E and Amadori D: Efficacy of endocrine therapy in relation to progesterone receptor and Ki67 expression in advanced breast cancer. Br Cancer Res Treat *152*: 57-65, 2015.
34 Yu KD, Jiang YZ, Hao S and Shao ZM: Molecular essence and endocrine responsiveness of estrogen receptor-negative, progesterone receptor-positive, and HER2-negative breast cancer. BMC Med *13*: 254, 2015.
35 Luoh SW, Ramsey B, Park B and Keenan E: Quantitative progesterone receptor expression and efficacy of anti-estrogen therapy in breast cancer. Breast J *20*: 46-52, 2014.
36 Harvey JM, Clark GM, Osborne GK and Allred DC: Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. J Clin Oncol *17*: 1474, 1999.
37 Elledge RM, Green S, Pugh R, Allred DC, Clark GM, Hill J, Ravdin P, Martino S and Osborne CK: Estrogen receptor ER and progesterone receptor PgR by ligand-binding assay compared with ER, PgR and pS2 by immunohistochemistry in predicting response to tamoxifen in metastatic breast cancer: A Southwest Oncology Group Study. Int J Cancer *89*: 111-117, 2000.
38 Mohsin SK, Weiss H, Havighurst T, Clark GM, Berardo M, Roanh LD, To TV, Zho Q, Love RR and Allred DC: Progesterone receptor by immunohistochemistry and clinical outcome in breast cancer: A validation study. Modern Pathol *17*: 1545–1554, 2004.
39 Chebil G, Bendahl PO, Idvall I and Ferno M: Comparison of immunohistochemical and biochemical assay of steroid receptors in primary breast cancer – Clinical associations and reasons for discrepancies. Acta Oncol *42*: 719-725, 2003.
40 Khoshnoud MR, Löfdahl B, Fohlin H, Fornander T, Stål O, Lambert Skoog L, Bergh J and Nordenskjöld B: Immunohistochemistry compared to cytosol assays for determination of estrogen receptor and prediction of the long-term effect of adjuvant tamoxifen. Br Cancer Res Treat *126*: 421-430, 2010.

Received February 4, 2016
Revised March 21, 2016
Accepted March 22, 2016