International Journal of Research and Review
Vol. 9; Issue: 12; December 2022
Website: www.ijrrjournal.com
Original Research Article *E-ISSN: 2349-9788; P-ISSN: 2454-2237*

Clinical Utility of Predictive Markers in Triple Negative Breast Cancer

Dr Hemangini Vora[1], Dr Kruti Rajvik[2], Dr Nupur Patel[3], Dr Sonia Parikh[4], Dr Priti Trivedi[5], Dr Shashank Pandya[6]

[1]Professor and Head, Cancer Biology Department, Head Immunohematology Lab, [2]Junior Scientific Officer Immunohematology Lab, [3]Research Assistant, Immunohematology Lab, [4]Professor Medical Oncology, [5]Professor and Head, Oncopathology, [6]Director, Professor and Head Surgical Oncology;
The Gujarat Cancer & Research Institute Medicity, Asarwa, Ahmedabad-380016

Corresponding Author: Dr Hemangini Vora

DOI: https://doi.org/10.52403/ijrr.20221219

ABSTRACT

Background & Objective: Triple negative breast cancer (TNBC) is an aggressive disease and visceral metastasis occurs due to unavailability of targeted therapy. This study was undertaken to evaluate incidence of predictive markers in TNBC which further guide to select adjuvant treatment.

Method: This study evaluated androgen receptor (AR), PI3K, mTOR, c-myc and TLE3 expression by immunohistochemistry on tumor tissues of 50 untreated TNBC. The protein expression of markers was correlated with clinicopathological parameters and disease status. The data was analysed statistically using SPSS software version 20.

Results: The incidence of expression of AR (18%) and TLE3 (22%) was found lower and c-myc (88%), PI3K (74%) and mTOR (86%) was found higher in TNBC. 82% were quadruple negative breast cancer. PI3K expression was seen higher in post-menopausal patients and mTOR expression was significantly higher in smaller tumor size and high BR score tumors with a trend in younger patients. Overexpression of c-myc was associated with post-menopausal status, early stage disease and high BR score tumors. These markers when intercorrelated, a significant positive correlation was noted of PI3K with mTOR, and mTOR with c-myc. Further, c-myc emerged as significant prognosticator predicting higher relapse rate in quadruple negative breast cancer, and death rate in total patients and quadruple negative breast cancer in multivariate analyses.

Interpretation & Conclusion: Identification of potentially actionable targets is essential to plan adjuvant therapy for better patient management of TNBC.

Key Words: predictive markers, c-myc, PI3K, mTOR, TNBC

INTRODUCTION

Triple negative breast cancer (TNBC) is characterized by a lack of estrogen receptor (ER), progesterone receptor (PR) and Her-2 neu receptor (Her-2). TNBC is typically an aggressive subtype of breast cancer seen in younger women with high tumor grade. TNBC does not respond to hormonal therapy or anti-Her2 therapy and only choice of treatment is cytotoxic chemotherapy. Approximately 30% of TNBC patients have higher risk of visceral metastasis which occurs within three years of diagnosis after standard adjuvant chemotherapy (1,2). Predictive marker profiling is of utmost importance which can potentially refine the use of therapy to improve disease-free and overall survival in TNBC. The present study aimed to evaluate clinical significance of certain predictive markers such androgen receptor (AR), c-myc, TLE3, PI3K and mTOR expression by immunohistochemistry in TNBC of western India thereby to identify which inhibitors can be incorporated in adjuvant

chemotherapy for better patient management.

MATERIALS AND METHODS

Patients

This retrospective study included 50 TNBC patients who had been diagnosed and treated at GCRI during 2014 to 2019 were included in the study. The detailed clinical history such as patient's age, menopausal status, disease stage, histopathological findings, treatment offered and disease status was recorded from the case files maintained at the Institutional Medical Record Department. Formalin fixed paraffin embedded tumor tissue (FFPE) blocks were collected from Histopathology department of the institute. Disease staging was done according to UICC TNM classification. Disease status was assessed by clinical examination, radiological investigations and biochemical investigations. The study was approved by Institutional Scientific Review Board and Ethics Committee. Patients other than TNBC subtype and subjected to neo-adjuvant therapy (either Radiotherapy or Chemotherapy before surgery) and patient with stage IV TNBC were excluded from the study.

Immunohistochemical localization

The 4µm thin sections were cut on microtome (Leica, Germany) and taken on 3-aminopropyl triethoxysilane (APES) coated slides. Immunohistochemical localization of AR, PI3K, mTOR, c-myc and TLE3 was performed on FFPE tissue blocks containing primary tumor and evaluated by Haematoxylene and Eosin (H&E) staining, on Ventana Benchmark XT autoimmunostainer using Ventana reagents (Ventana, USA). Briefly, the protocol includes following steps of deparaffinization using EZ solution, antigen retrieval using cell conditioning (CC1), incubation with ultra view DAB inhibitor for 4 minutes, 100µl of primary antibody, ultra view HRP multimer for 8 minutes, ultra view DAB detection kit for 8 minutes, counterstain with haematoxylin for 8 minutes, bluing reagent for 4 minutes and mounted with DPX. The primary antibody clone, company, dilution, antigen retrieval time and antibody dilution used are as follows:

Primary antibody	Clone	Company	Dilution	Cell Conditioning	Primary antibody incubation time (mins)
AR	SP107	Cell Marque	1:100	Mild	32
PI3K	SP62	Sigma	1:100	Standard	32
mTOR	215Q18	Invitrogen	1:100	Standard	32
c-myc	9E10	Invitrogen	1:100	Mild	32
TLE3	CL3575	Invitrogen	1:30	Standard	32

Scoring

Two independent observers familiar with immunohistochemistry and unaware of the clinical outcome scored all the sections. The sections were scored with semi quantitative scoring ranging from negative (no staining) to 3+ (1+: staining in <10% of cells, 2+: staining in 10% to 50% of cells, and 3+: staining in >50% of cells) For statistical evaluation, scores 1+, 2+ and 3+ were taken together as positive group.

STATISTICAL ANALYSIS

Statistical analysis was carried out using SPSS statistical software version 20 (SPSS Inc, USA). Pearson's Chi-square test with Pearson's correlation coefficient (r) was used to assess correlation and significance between the two parameters. In case of patient number less than 5 in the cells of 2 x 2 tables, Yates' Continuity Correction value along with its significance was taken into consideration. Univariate survival analysis was carried out by Kaplan and Meier method and Log Rank statistics was used to assess the prognostic significance of disease free survival (DFS) and overall survival (OS). Multivariate survival analysis was performed using Cox regression model with forward stepwise (likelihood ratio) method. The Wald statistics and relative risk [Exp(B)] with 95% confidence interval (CI) for Exp(B) were used to evaluate the prognostic significance. P values ≤0.05

were considered significant.

RESULTS

Androgen receptor (AR) expression

Nuclear expression of AR was noted in 18% of TNBC tumors with an intensity of 1+, 2+ and 3+ in 10%, 4%, and 4% of the patients, respectively (Figure 1a,b). The incidence of quadruple negative breast cancer (TNBC lacks AR) was observed 82%. In relation to clinicopathological features, a trend of reduced AR expression was noted in high BR score tumors and histologic grade III tumors (Table 1).

PI3K expression

Cytoplasmic expression of PI3K was noted in 74% of TNBC tumors with an intensity of 1+, 2+ and 3+ in 2%, 30%, and 42% of the patients, respectively (Figure 1c). In relation to clinicopathological features, a trend of higher PI3K expression was noted in patients with postmenopausal status (Table 1).

mTOR expression

Cytoplasmic expression of mTOR was noted in 86% of TNBC tumors with an intensity of 1+, 2+ and 3+ in 4%, 40%, and 42% of the patients, respectively (Figure 1d). In relation to clinicopathological features, a significant higher mTOR expression was noted in patients with smaller tumor size (T1 or T2, P=0.01), and high BR score tumors (P=0.01) and a similar trend was noted in patients with age $\geq$ 45 years and histologic grade III tumors (Table 1).

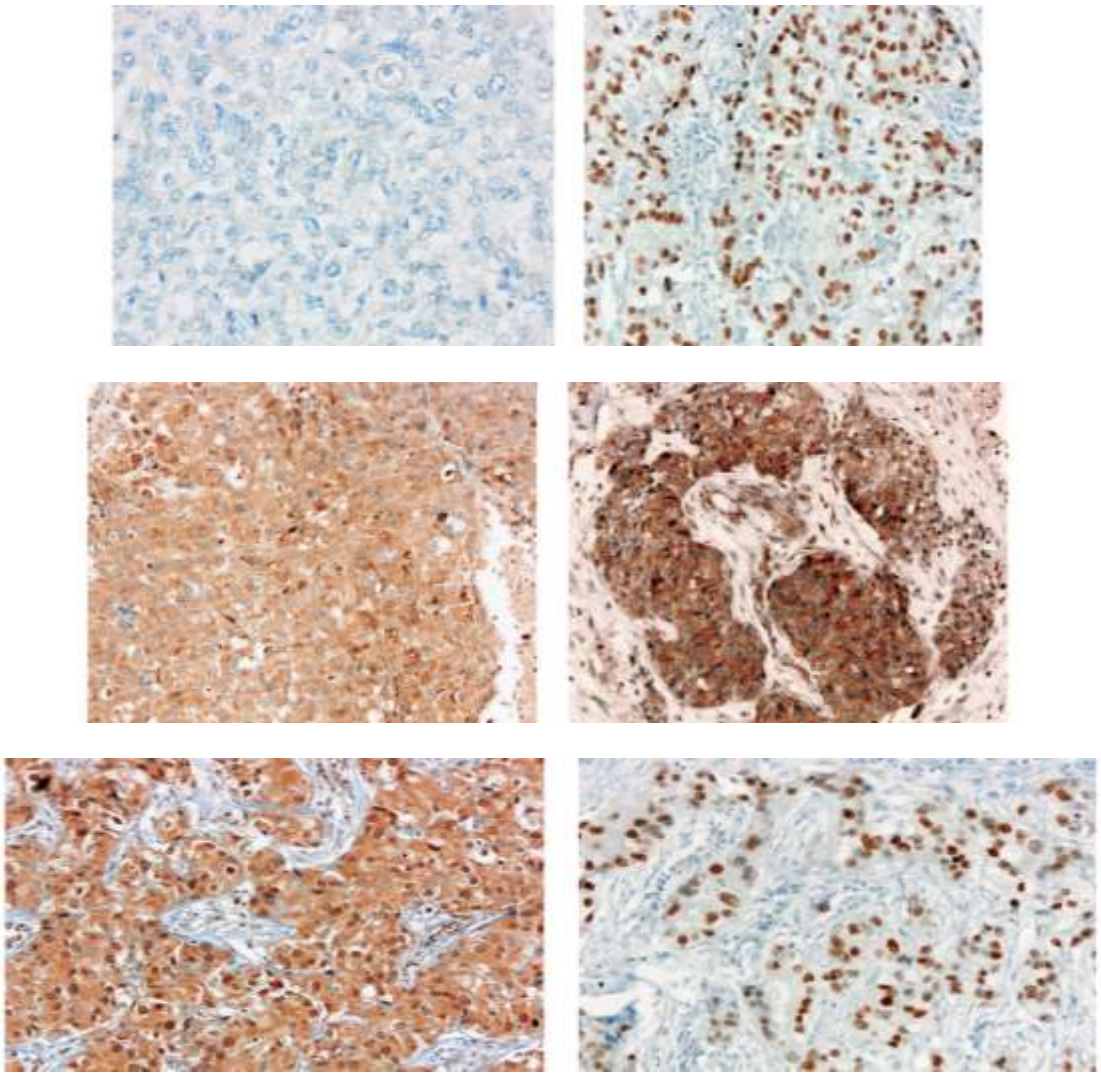

Figure 1 (a-f): Immunohistochemical staining of Negative Control (a), AR (b), PI3K (c), mTOR (d), c-myc (e), and TLE3 (f).

Table 1: Relationship between predictive markers and clinicopathological parameters in TNBC

Parameters	N (%)	Androgen Receptors N (%)	PI3K Positive N (%)	m-TOR Positive N (%)	c-myc Positive N (%)	TLE3 Positive N (%)
Total patients	50	09 (18)	37 (74)	43 (86)	44 (88)	11 (22)
Age (Years)						
<45	38 (76)	06 (16)	26 (26)	31(82)	33 (87)	26 (26)
≥45	12 (24)	03 (25)	11 (92)	12 (100)	11 (92)	01 (08)
Menopausal Status						
Premenopausal	20 (40)	04 (20)	15 (75)	16 (80)	15 (75)	06 (30)
Postmenopausal	30 (60)	05 (17)	22 (73)	27 (90)	29 (97) [c]	05 (17)
Tumor size						
T1 + T2	43 (86)	08 (19)	32 (74)	39 (91)[a]	40 (93)[d]	10 (23)
T3 + T4	07 (14)	01 (14)	05 (71)	04 (57)	04 (57)	01 (14)
Lymph node Status						
Negative	27 (54)	04 (15)	19 (70)	22 (82)	23 (85)	06 (22)
Positive	23 (46)	05 (22)	18 (78)	21 (91)	21 (91)	05 (22)
Stage						
Early (IIA + IIB)	36 (72)	06 (17)	26 (72)	32 (89)	34 (94)[e]	09 (25)
Advance (IIIA + IIIB)	14 (28)	03 (21)	11 (79)	11 (79)	10 (71)	02 (14)
Histopathology Type						
IDC	48 (96)	09 (19)	35 (73)	42 (88)	42 (88)	10 (21)
IDC + DCIS	02 (04)	00 (00)	02 (100)	01 (50)	02 (100)	01 (50)
BR Score	43	08 (19)	32 (74)	37 (86)	39 (91)	09 (21)
Low (score 4-7)	23(54)	06 (26)	16 (70)	17 (74)	19 (83)	05 (22)
High (score 8-9)	20 (46)	2 (10)	16 (80)	20 (100)[b]	20 (100)[f]	04 (20)
Histology Grade	41	07 (17)	30 (73)	35 (85)	38 (93)	07 (17)
Low grade (Grade I)	20 (49)	04 (20)	14 (70)	15 (75)	18 (90)	03 (15)
High grade (Grade II+III)	21 (51)	03 (14)	16 (76)	20 (95)	20 (95)	04 (19)
Metastasis						
Remission	36 (72)	08 (22)	27 (75)	31 (86)	33 (92)	09 (25)
Relapse	14 (28)	01 (07)	10 (71)	12 (86)	11 (78)	02 (14)

IDC: infiltrating ductal carcinoma; DCIS: Ductal carcinoma in situ
a: $\chi 2$=5.63, r=-0.03, p=0.01; b: $\chi 2$= 6.06, r=0.37, p=0.014; c: $\chi 2$=5.33, r=0.32, p=0.02; d: $\chi 2$=7.3, r=-0.38, p=0.007; e: $\chi 2$=5.05, r=-0.31, p= 0.02; f: $\chi 2$=3.83, r=0.299, p=0.05;

C-myc expression

Cytoplasmic and nuclear expression of c-myc was noted in 88% of TNBC tumors with an intensity of 1+, 2+ and 3+ in 24%, 46%, and 18% of the patients, respectively (Figure 1e). In relation to clinicopathological features, a significant higher expression of c-myc was noted in patients with postmenopausal status (P=0.02), smaller tumor size (T1 or T2, P=0.007), stage II disease (P=0.02) and high BR score tumors (P=0.05, Table 1).

TLE3 expression

Nuclear expression of TLE3 was noted in 22% of TNBC tumors with an intensity of 1+, 2+ and 3+ in 2%, 4%, and 16% of the patients, respectively (Figure 1f). In relation to clinicopathological features, a trend of higher TLE3 expression was noted in patients with age <45 years, postmenopausal status, and stage II disease (Table 1).

Marker expression and disease metastasis

In patients who did not developed metastasis showed a trend of higher expression of AR and c-myc as compared to their counterparts (Table 1).

Survival Analysis

In univariate Kaplan & Meier survival analysis for disease free survival, a trend of higher relapse rate was noted in c-myc negative TNBC. In quadruple negative breast cancer, a significant higher relapse rate was noted in c-myc negative TNBC than c-myc positive TNBC (Log rank=6.47, df=1, P=0.01, Figure 2, Table 2a). For overall survival, a significant higher death rate was noted in c-myc negative TNBC than c-myc positive TNBC in total patients and quadruple negative breast cancer (Table 2b). In multivariate analysis by Cox regression forward step wise model, no expression of c-myc emerged as significant prognosticator predicting higher relapse rate

in quadruple negative breast cancer (Wald=5.31, df=1, P=0.02, Exp(B)=0.21), and higher death rate in total patients (Wald=6.16, df=1, P=0.01, Exp(B)=0.13), and quadruple negative breast cancer (Wald=7.17, df=1, P=0.007, Exp(B)=0.11).

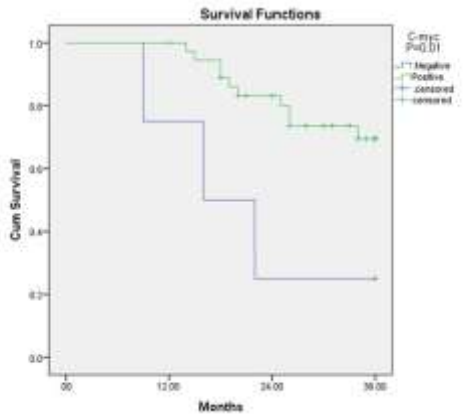

Figure 2: Kaplan & Meier survival analysis indicated that no expression of c-myc associated with reduced disease free survival.

Table 2a: Univariate survival analysis for disease free survival.

Markers	N	No. of patients relapsed	Log rank	df	P value
Androgen negative	41	13 (32)	1.24	1	0.26
Androgen positive	9	01 (11)			
PI3K negative	13	04 (31)	0.09	1	0.76
PI3K positive	37	10 (27)			
mTOR negative	7	02 (29)	0.00	1	0.92
mTOR positive	43	12 (28)			
c-myc negative	6	03 (50)	2.72	1	0.09
c-myc positive	44	11 (25)			
TLE3 negative	39	12 (31)	0.45	1	0.50
TLE3 positive	11	02 (18)			
QTNBC c-myc negative	4	03 (75)	6.47	1	0.01
QTNBC c-myc postive	37	10 (27)			

Table 2b: Univariate survival analysis for overall survival.

Markers	N	No. of patients died	Log rank	df	P value
Androgen negative	41	06 (15)	1.30	1	0.25
Androgen positive	9	00 (00)			
PI3K negative	13	02 (16)	0.07	1	0.78
PI3K positive	37	04 (11)			
mTOR negative	7	02 (29)	1.31	1	0.25
mTOR positive	43	04 (10)			
c-myc negative	6	03 (50)	8.64	1	0.003
c-myc positive	44	03 (07)			
TLE3 negative	39	05 (13)	0.11	1	0.73
TLE3 positive	11	01 (10)			
QTNBC c-myc negative	4	03 (75)	10.63	1	0.001
QTNBC c-myc postive	37	03 (09)			

Intermarker correlation

The marker when intercorrelated significant positive correlation was noted between PI3K and mTOR (r=+0.28, P=0.044), and mTOR and c-myc (r=+0.56, P=0.0001).

DISCUSSION

Gene sequencing and protein profiling have contributed significantly in the characterization of molecular landscape of primary breast cancer (3,4). The gene mutation profiling of larger patient series of TNBC (N=760) observed most frequent mutation in TP53 (64%), PI3KCA (13%) and PTEN (4%). The frequency of other gene mutations was less than 0.5% and therefore not considered significant in TNBC. In addition, TNBC metastatic tissues had significantly higher incidence of gene mutations than primary TNBCs. Also, targetable mutations PI3KCA and EGFR, and protein expression of AR, Ki-67, PTEN

and topoisomerase 1 were found (5). The present study evaluated incidence of protein expression of predictive markers such as AR, PI3K, mTOR, c-myc and TLE3 in TNBC of western part of India. A lower incidence of expression of AR (18%) and TLE3 (22%), with a higher incidence of c-myc (88%), PI3K (74%) and mTOR (86%) was observed in TNBC. The incidence of quadruple negative breast cancer found was 82%.

PI3K/AKT/mTOR pathway is an intracellular signal transduction pathway that promotes metabolism, proliferation, cell survival, growth and angiogenesis in response to extracellular growth factor signals such as TGF-β and EGF and found frequently altered in various cancers. Increased signaling of PI3K/AKT/mTOR pathway is noted in breast cancer including TNBC. C-myc is the downstream molecule of this pathway and of Notch signaling pathway which is a nuclear DNA binding protein belongs to an oncogene family involved in cell cycle regulation. It promotes cell proliferation, immortalization, dedifferentiation and transformation. It was noted that Myc family oncogene and its protein product is deregulated in >50% of human cancers which is associated with poor prognosis. In the current study, 3/4th of TNBC exhibited higher expression of PI3K, mTOR and c-myc. PI3K expression was higher in post-menopausal patients, and mTOR expression was significantly higher in smaller tumor size and high BR score tumors with a trend in younger patients. Overexpression of c-myc was associated with post-menopausal status, early stage disease and high BR score tumors. These markers when intercorrelated, a significant positive correlation was noted of PI3K with mTOR, and mTOR with c-myc. The Cancer Genome Atlas project on large scale genomic analysis of 65 basal like TNBC depicted high activation of Tp53 mutations, loss of RB1 and BRCA1, high activation of PI3K pathway, and hyperactivation of FOXM1. High activation of PI3K pathway either through PIK3CA gene mutation or loss of negative regulators INPP4B and/or PTEN was noted (6). PI3K/AKT/mTOR pathway is one of the important and active pathways involved in chemoresistance and survival of TNBC. This pathway has been considered as a potential molecular target for the design of therapeutic agents to treat TNBC. Further, pharmacological inhibition of the PI3K/AKT/mTOR pathway markedly decreased Myc level and exhibited remarkable therapeutic efficacy in Myc-driven cancers, including neuroblastoma, small-cell lung carcinoma, breast cancer, and multiple hematopoietic cancers (7-11).

AR expression was noted in 18% of TNBC and high grade tumors showed reduced expression. Few other studies noted in the range of 13%-37% of TNBC and had older age at presentation (12-14). The prognostic significance of AR expression is contradictory as some studies have shown AR expression has been associated with both favorable and other studies have shown poor prognoses (14-18). AR-positive TNBC has a lower Ki-67 index than AR-negative TNBC and could therefore be less sensitive to chemotherapy (19). In our study majority of AR expressing tumors of the patients showed disease remission except one patient. A study by Xiu et al observed AR+ TNBC is younger and showed higher TLE3 expression (20) which is not observed in the present study. Further AR+ TNBC showed significantly high mutation rate of PIK3CA, AKT1 and ERBB2.

Regarding TLE3 expression, it was observed in 22% of TNBC with higher expression in younger, post-menopausal and early stage TNBC. A study by Kashiwagi et al observed significantly higher TLE3 expression in TNBC (58%) than non-TNBC (41%) and TLE3 expression emerged as a significant prognosticator predicting better PFS in univariate and multivariate analysis suggesting useful marker predicting therapeutic effect of eribulin chemotherapy in TNBC (21). The TLE3 expression has also been reported to be involved in the therapeutic effect of taxane. TLE3 gene is a member of the Notch signal transduction

pathway, which inhibits transcriptional activation (22-23) and Notch is involved in the maintenance of the stemness of stem cells as well as cancer stem cells has been reported in some cancers including brain tumors and breast cancer (24).

In survival analysis, no expression of c-myc emerged as significant prognosticator predicting higher relapse rate in quadruple negative breast cancer, and death rate in total patients and quadruple negative breast cancer in multivariate analyses suggesting c-myc negative TNBC is molecularly undifferentiated TNBC which did not respond to conventional treatments.

CONCLUSION

75% of TNBCs express targetable proteins of PI3K-mTOR pathway indicate PI3K inhibitors and mTOR inhibitors use as adjuvant treatment. C-myc is a key mediator of Notch signaling pathways and 88% TNBCs express c-myc are the candidates for Notch inhibitors. Only one fifth express TLE3 which guides the use of taxanes. AR is emerging as an important new target and AR positive TNBCs might be benefitted with AR inhibitors. Thus, biomarker profiling identifies potentially actionable targets which guide selection of adjuvant treatment modalities for TNBC to improve patient's survival.

Declaration by Authors

Ethical Approval: Approved

Acknowledgement: None

Source of Funding: The Gujarat Cancer Society (EC/20/2018)

Conflict of Interest: The authors declare no conflict of interest.

REFERENCES

1. Craig DW, O'Shaughnessy JA, Kiefer JA, Aldrich J, Sinari S, Moses TM, Wong S, Dinh J, Christoforides A, Blum JL, Aitelli CL. Genome and Transcriptome Sequencing in Prospective Metastatic Triple-Negative Breast Cancer Uncovers Therapeutic Vulnerabilities Genome Sequencing in Recurrent Triple-Negative Breast Cancer. Molecular cancer therapeutics. 2013 Jan 1;12(1):104-16.
2. Schmadeka R, Harmon BE, Singh M. Triple-negative breast carcinoma: current and emerging concepts. American journal of clinical pathology. 2014 Apr 1;141(4):462-77.
3. Turner NC, Reis-Filho JS. Tackling the diversity of triple-negative breast cancer. Clinical Cancer Research. 2013 Dec 1;19(23):6380-8.
4. Hansen AR, Bedard PL. Clinical application of high-throughput genomic technologies for treatment selection in breast cancer. Breast Cancer Research. 2013 Oct;15(5):1-0.
5. Millis SZ, Gatalica Z, Winkler J, Vranic S, Kimbrough J, Reddy S, O'Shaughnessy JA. Predictive biomarker profiling of> 6000 breast cancer patients shows heterogeneity in TNBC, with treatment implications. Clinical breast cancer. 2015 Dec 1;15(6):473-81.
6. The Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012 Sep 23;490(7418):61-70.
7. Bjornsti MA, Houghton PJ. The TOR pathway: a target for cancer therapy. Nature Reviews Cancer. 2004 May;4(5):335-48.
8. Frost P, Moatamed F, Hoang B, Shi Y, Gera J, Yan H, Frost P, Gibbons J, Lichtenstein A. In vivo antitumor effects of the mTOR inhibitor CCI-779 against human multiple myeloma cells in a xenograft model. Blood. 2004 Dec 15;104(13):4181-7.
9. Chapuis N, Tamburini J, Green AS, Vignon C, Bardet V, Neyret A, Pannetier M, Willems L, Park S, Macone A, Maira SM. Dual Inhibition of PI3K and mTORC1/2 Signaling by NVP-BEZ235 as a New Therapeutic Strategy for Acute Myeloid Leukemia Antileukemic Activity of the NVP-BEZ235 Compound in AML. Clinical Cancer Research. 2010 Nov 15;16(22): 5424-35.
10. Yu K, Toral-Barza L, Discafani C, Zhang WG, Skotnicki J, Frost P, Gibbons JJ. mTOR, a novel target in breast cancer: the effect of CCI-779, an mTOR inhibitor, in preclinical models of breast cancer. Endocrine-related cancer. 2001 Sep 1;8(3):249-58.
11. Swords RT, Schenk T, Stengel S, Gil VS, Petrie KR, Perez A, Ana R, Watts JM,

Vargas F, Elias R, Zelent A. Inhibition of the PI3K/AKT/mTOR pathway leads to down-regulation of c-Myc and overcomes resistance to ATRA in acute myeloid leukemia. Blood. 2015 Dec 3;126(23):1363.

12. Mina A, Yoder R, Sharma P. Targeting the androgen receptor in triple-negative breast cancer: current perspectives. OncoTargets and therapy. 2017;10:4675.
13. Cauley JA, Lucas FL, Kuller LH, Stone K, Browner W, Cummings SR, Study of Osteoporotic Fractures Research Group. Elevated serum estradiol and testosterone concentrations are associated with a high risk for breast cancer. Annals of internal medicine. 1999 Feb 16;130(4_Part_1):270-7.
14. Zakaria F, El-Mashad N, Mohamed D. Androgen receptor expression as a prognostic and predictive marker in triple-negative breast cancer patients. Alexandria journal of medicine. 2016 Jun 8;52(2):131-40.
15. Qu Q, Mao Y, Fei XC, Shen KW. The impact of androgen receptor expression on breast cancer survival: a retrospective study and meta-analysis. PloS one. 2013 Dec 4;8(12):e82650.
16. Tang D, Xu S, Zhang Q, Zhao W. The expression and clinical significance of the androgen receptor and E-cadherin in triple-negative breast cancer. Medical oncology. 2012 Jun;29(2):526-33.
17. Choi JE, Kang SH, Lee SJ, Bae YK. Androgen receptor expression predicts decreased survival in early stage triple-negative breast cancer. Annals of surgical oncology. 2015 Jan;22(1):82-9.
18. Aleskandarany MA, Abduljabbar R, Ashankyty I, Elmouna A, Jerjees D, Ali S, Buluwela L, Diez-Rodriguez M, Caldas C, Green AR, Ellis IO. Prognostic significance of androgen receptor expression in invasive breast cancer: transcriptomic and protein expression analysis. Breast cancer research and treatment. 2016 Sep;159(2):215-27.
19. Barton VN, D'Amato NC, Gordon MA, Christenson JL, Elias A, Richer JK. Androgen receptor biology in triple negative breast cancer: a case for classification as AR+ or quadruple negative disease. Hormones and cancer. 2015 Dec;6(5):206-13.
20. Xiu J, Obeid E, Gatalica Z, Reddy S, Goldstein LJ, Link J, *et al.* Biomarker comparison between androgen receptor positive triple negative breast cancer (AR+ TNBC) and quadruple negative breast cancer. Abstract. Cancer Res DOI: 10.1158/1538-7445.SABCS15-P3-07-26 Published February 2016.
21. Kashiwagi S, Fukushima W, Asano Y, Goto W, Takada K, Noda S, Takashima T, Onoda N, Ohsawa M, Hirakawa K, Ohira M. Identification of predictive markers of the therapeutic effect of eribulin chemotherapy for locally advanced or metastatic breast cancer. BMC cancer. 2017 Dec;17(1):1-0.
22. Leon C, Lobe CG. Grg3, a murine Groucho-related gene, is expressed in the developing nervous system and in mesenchyme-induced epithelial structures. Developmental dynamics: an official publication of the American Association of Anatomists. 1997 Jan;208(1):11-24.
23. Whitfield ML, Sherlock G, Saldanha AJ, Murray JI, Ball CA, Alexander KE, Matese JC, Perou CM, Hurt MM, Brown PO, Botstein D. Identification of genes periodically expressed in the human cell cycle and their expression in tumors. Molecular biology of the cell. 2002 Jun 1;13(6):1977-2000.
24. Androutsellis-Theotokis A, Leker RR, Soldner F, Hoeppner DJ, Ravin R, Poser SW, Rueger MA, Bae SK, Kittappa R, McKay RD. Notch signalling regulates stem cell numbers in vitro and in vivo. Nature. 2006 Aug;442(7104):823-6.

How to cite this article: Hemangini Vora, Kruti Rajvik, Nupur Patel et.al. Clinical utility of predictive markers in triple negative breast cancer. *International Journal of Research and Review*. 2022; 9(12): 179-186. DOI: *https://doi.org/10.52403/ijrr.20221219*

******