Oncology
and Radiotherapy ©
1 (52) 2020: 001-006 • Research Article

Role of COX-2 inhibitors as maintenance therapy in non-metastatic triple negative breast cancer Egyptian patients, single institution study

Fatma Gharib[1], Yomna Zamzam[2], Lamiss Mohamed Sad[1]

[1] Department of Clinical Oncology and Nuclear Medicine, Tanta University, Egypt
[2] Department of Pathology, Tanta University, Egypt

SUMMARY

Objective: In comparison to other breast subtypes, Triple Negative Breast Cancer (TNBC) has worst prognosis. In this study, we evaluate the role of maintenance COX-2 inhibitors in non-metastatic TNBC patients.

Methods: This study was a prospective, open-label, 1:1-ratio, randomized trial. Eighty-six patients diagnosed with non-metastatic TNBC and proved to have positive COX-2 expression by immunohistochemically staining were enrolled in this trial. The patients were treated and followed up at Tanta University Hospitals in the period from January 2014 to October 2019. Patients randomized to receive COX-2 inhibitors (Celecoxib 200 mg once daily) as maintenance therapy after completion of adjuvant therapy till progression or toxicity (arm A) or observation without maintenance therapy (arm B) in ratio of 1 to 1. The primary endpoint was Disease Free Survival (DFS). Positive COX-2 expression was defined as more than 10% cytoplasmic staining.

Results: With median follow up of 59 months, the 4-year DFS rate was significantly higher for patients received celecoxib compared to patients who didn't receive maintenance therapy (66% and 41.9% respectively) p<0.001.

Worse DFS were associated with conservative breast surgery, high Ki-67 and positive family history of breast cancer respectively (p<0.001, 0.016 and 0.002 respectively).

Surgical approach and use of Cox-2 inhibitors remained statistically significant with DFS in multivariate analysis [p=0.001; 95%CI (0.071-0.508); HR=0.190) and p=0.006; 95%CI (1.466-10.27); HR=3.880) respectively].

Conclusion: This study showed that use of COX-2 inhibitors as maintenance therapy based on COX-2 expression may be associated with better DFS for non-metastatic TNBC patients. So, targeting COX-2 may have a role in this aggressive disease..

Key words: celecoxib, TNBC, COX-2 expression

Address for correspondence:

Lamiss Mohamed Sad, Department of Clinical Oncology and Nuclear Medicine, Tanta University, Egypt, email: lamissmohamed2@yahoo.com, lamissmohamed@med.tanta.edu.eg

Word count: 2965 **Tables:** 02 **Figures:** 03 **References:** 31

Received: - 26 January, 2020

Accepted: - 24 March, 2020

Published: - 12 May, 2020

INTRODUCTION

Triple-Negative Breast Cancer (TNBC) is characterized by negativity of Estrogen Receptor (ER), Progesterone Receptor (PR), and Human Epidermal Growth Factor Receptor 2 (HER2). Luminal androgen receptor, mesenchymal, basal-like immunosuppressed, and basal-like immune-activated are four TNBC subtypes with different molecular behaviours [1]. Triple-negative breast cancer is a rapid growing than other types of tumour and behaves more aggressively breast cancer with a poorer prognosis. TNBC is characterized by higher relapse rates compared with ER-positive breast cancers. The risk of loco regional recurrence and distant metastases especially lung and brain involvement are more obvious in TNBC [2-5]. Due to absence of expression of ER, PR and the HER2 protein, endocrine therapy and anti-HER2 are ineffective in the treatment of triple negative breast cancer [6]. Despite using antiandrogen in the era of triple negative breast cancer its role remains inconclusive [7].

Although the efficacy of immunotherapy in the last years of research as proved many authors where they classify the triple negative breast cancer depending on immune marker expression [8, 9], but the role of COX 2 remains to be elucidated as potential target.

The conversion of arachidonic acid to prostaglandins is catalysed by Cyclooxygenase (COX). The inductive prostaglandin, COX-2 was not associated with inflammation only but also new vessel formation, tumour progression, inhibition of apoptosis and development of metastases [10, 11]. Over expression of COX-2 was reported in a variety of cancers of them primary breast cancer [12]. Studies revealed that among different breast cancer subtypes, COX-2 is highly expressed in TNBC [13]. Large tumour size and higher grade are associated with ER-/PR-negative [13-15]. Being TNBC and COX-2 expression are associated with worse outcome in early and locally advanced breast cancer patients [16-19]. So, we are going to study the role of COX 2 as maintenance therapy in non-metastatic triple negative breast cancer.

PATIENTS AND METHODS

This is a prospective randomized (1:1-ratio) trial. Eighty-six patients of non-metastatic TNBC were involved in this study. The patients were treated and followed up at Tanta University Hospitals through the period from January 2014 to October

2019. Adjuvant, neoadjuvant and postoperative radiotherapy were administrated according to guidelines [20].

This trial was approved by Research Ethics Committee of Tanta University. A written informed consent was received from all patients. Women aged more than eighteen years were considered were eligible if they had histopathologically proved TNBC (ER-negative, PR-negative and HER2 negative) with positive COX-2 expression ≥ 10% by immunohistochemically staining. The main exclusion criteria; evidence of distant metastases, hormonal receptor positive or HER2 enriched breast cancer, asynchronous bilateral breast cancer or COX-2 expression <10%.

In this study, patients received either COX-2 inhibitors (celecoxib) 200 mg once daily as maintenance therapy till progression or toxicity (arm A) or observation without maintenance therapy (Arm B) after completion of adjuvant chemotherapy and/or radiotherapy (Figure 1).

Patients received adjuvant or neoadjuvant in the form of AC (Day 1) Doxorubicin 60mg/m^2- Cyclophosphamide 600 mg/m^2 IV over 30 minutes. Repeat cycle every 21 days for 4 cycles, followed by Day 1: Paclitaxel 80 mg/m^2 by 1-hour IV infusion weekly for 12 weeks.

Immunohistochemistry

Formalin-fixed, paraffin-embedded specimens were pathologically examined for the immunohistochemical studies for ER, PR, Her2 and Ki 67.

Four to five µm thick, formalin fixed, paraffin embedded tissue was immunohistochemical stained for COX-2 expression using avidin-biotin-peroxidase complex method. In summary, after dewaxing and hydration, endogenous peroxidase was blocked with 3% hydrogen peroxide. The pn, slides were incubated with rabbit monoclonal primary anti-COX2 antibody (Clone SP21, Cell Marque, Rocklin, California, USA, dilution 1:50). The slides were counter-stained by haematoxylin. Replacing the primary antibody with primary buffers and normal mouse or rabbit serum to establish primary control. Positive control was established using colon cancer. The immunostained sections were evaluated at a standard light microscope [21]. Positive expression was defined as COX-2 cytoplasmic staining ≥ 10% of tumour (Figure 2).

Statistical analysis

SPSS software version 20 was used for statistical analysis. Chi-square test was used to assess the difference between groups. Using Kaplan-Meier curves and log-rank test was used to assess survival. Statistical significance was considered for p value of <0.05 [22].

Study endpoints

Disease free survival was calculated from time of start of study to local recurrence, distant metastasis, contra lateral or ipsilateral breast tumour or death from any cause, whichever occurred first.

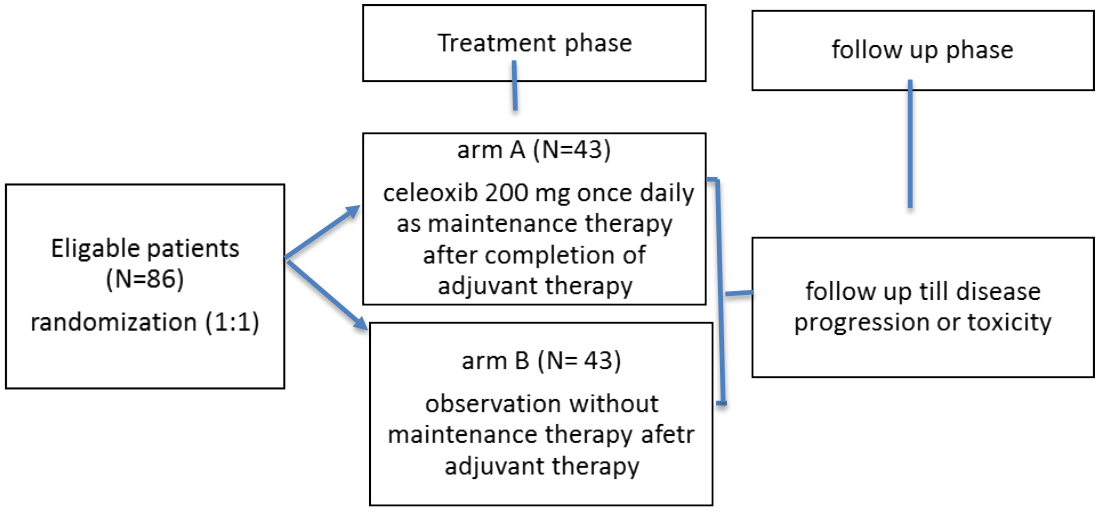

Fig. 1. Study flow

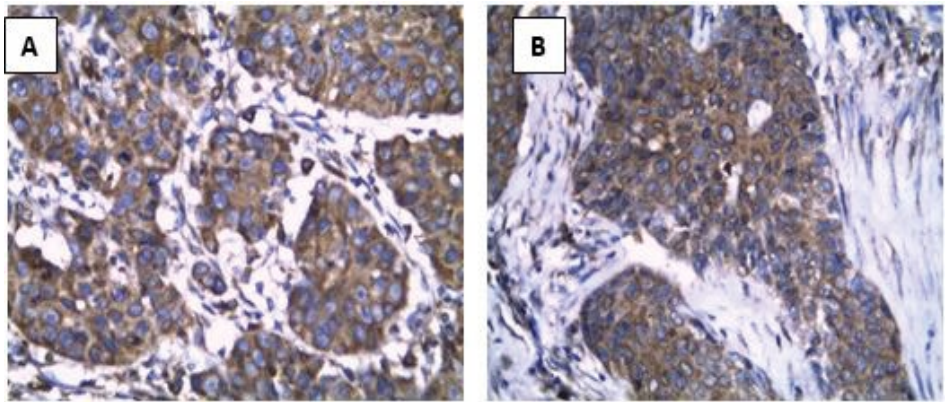

Fig. 2. (A): COX-2 expression in >10% of the tumour cells (COX-2 immunohistochemistry, x400); (B): COX-2 expression in >10% of the tumour cells in another case (COX-2 immunohistochemistry, x400)

Tab. 1. Patient criteria

Characteristics of all population	Arm (A) 43 (%)		Arm (B) 43 (%)		Total 86 (%)	
Age group						
≤ 48	19	44.2	15	34.9	34	39.5
>48	24	55.8	28	65.1	52	60.5
Menopausal status						
Premenopausal	23	53.5 46.5	22	51.2	45	52.3
Postmenopausal	20		21	48.8	41	47.7
LN						
N0	17	39.5	10	23.3	27	31.4
N1	13	30.2	20	46.5	33	38.4
N2	9	20.9	10	23.3	19	22.1
N3	4	9.3	3	7	7	8.1
Stage						
I	2	4.7	3	7	5	5.8
II	32	74.4	23	53.5	55	64
III	9	20.9	17	39.5	26	30.2
Tumor grade						
G1	6	14	5	11.6	11	12.8
G2	30	69.8	29	67.4	59	68.6
G3	7	16.3	9	20.9	16	18.6
Neo-adjuvant chemotherapy						
Yes	10	23.3	10	23.3	20	23.3
No	33	76.7	33	76.7	66	76.7
Surgery						
CBS	15	34.9	26	60.5	41	47.7
MRM	28	65.1	17	39.5	45	52.3
Ki 67						
Low	28	65.1	18	41.9	46	53.5
High	15	34.9	25	58.1	40	46.5
Family history						
No	33	76.7	26	60.5	59	68.6
Yes	10	23.3	17	39.5	27	31.4

Follow ups were every four months to report recurrence and death.

RESULTS

This prospective study included 86 patients diagnosed pathologically to have non-metastatic TNBC. The median age was 48 years (range, 28-60 years). Forty-five patient (52.3%) were premenopausal. Most of patients (68.6%) had lymph node involvement. Fifty-five (64%) of cases represented with stage II.

Regarding to arm (A), most of patients were premenopausal. Stage II and grade II were reported in 74.4% and 69.8% respectively. Ten patients received neoadjuvant chemotherapy and 28 patients (65.1%) underwent Modified Radical Mastectomy (MRM). Nearly similar criteria in arm (B), most of patients were premenopausal. Stage II and grade II were reported in 53.5.4% and 67.4% respectively. Ten patients received neoadjuvant chemotherapy and 17 patients (52.3%) underwent MRM (Table 1).

Disease free survival

Survival outcomes: With median follow up of 59 months, the 4- year DFS rate was 51.1%. Thirty-two patients had disease progression [7 cases (16.3%) for arm A and 25 (58.1%) for arm B]. The 4-year DFS rate was significantly higher in arm A than arm B (66% and 41.9%respectively) (p<0.001, HR=5.84; 95% confidence Interval=2.477:13.810). Correlation of DFS to the other prognostic features was clarified in (Figure 3).

In Univariate analysis; the factors associated with worse disease-free survival were conservative breast surgery, high Ki-67 and positive family history of breast cancer. Interestingly, no significant correlation was detected between tumour stage, lymph node involvement or neoadjuvant chemotherapy and DFS. Surgical approach and use of Cox-2

inhibitors remained statistically significant with DFS in multivariate analysis [p=0.001; 95%CI (0.071-0.508); HR=0.190) and p=0.006; 95%CI (1.466-10.27); HR=3.880) respectively] (Table 2).

DISCUSSION

Due to lack of treatment options of TNBC and their dismal prognosis, there is a need for new pathological marker such as COX-2 to give new hope for these patients [23].

There is established role of selective COX-2 inhibitors in reducing breast cancer as risk by promoting apoptosis, inhibiting cell proliferation and angiogenesis through decreased prostaglandin synthesis [24, 25]. The role of COX-2 inhibitors as maintenance therapy for patients with non-metastatic TNBC after adjuvant therapy wasn't discussed in previous clinical studies.

Several phase II trials revealed that the combination of celecoxib

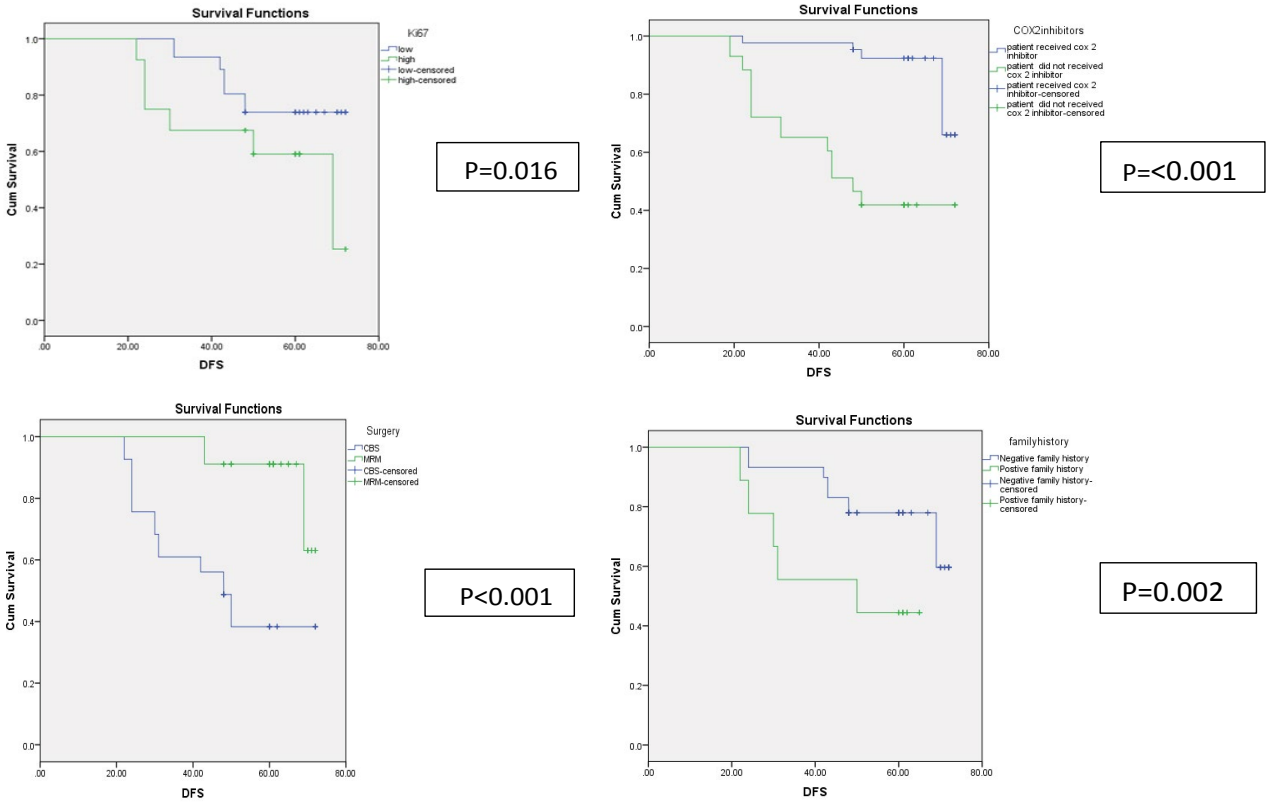

Fig. 3. Correlation of DFS and prognostic features

Tab. 2. The effects of clinic pathologic features on disease free survival by univariate and multivariate analysis

	Univariate			Multivariate		
	4-year DFS (%)	**95% CI**	**p**	**HR**	**95% CI**	**p**
Age group						
≤ 48	36.8					
>48	63.5	0.488-2.005	0.978			
Menopausal status						
Premenopausal	31.1	0.268-1.155	0.076			
Postmenopausal	73.2					
LN						
N0	76.9					
N1	44.8	0.761-1.585	0.062			
N2	43.8					
N3	57.1					
Stage						
I	60					
II	55.6	0.532-2.278	0.110			
III	57.7					
Tumor grade						
G1	45.5	0.415-1.698	0.183			
G2	55.7					
G3	62.5					
Neo-adjuvant chemotherapy						
Yes	60					
No	53.3	0.280-1.447	0.331			
Surgery						
CBS	38.3	0.084-0.423	<0.001	0.190	0.071-.0.508	0.001*
MRM	63.1					
Cox-2						
Yes	66.4		<0.001	3.880	1.466-10.27	0.006*
No	41.9					
Ki 67						
<15%	73.9	1.123-4.174	0.016	1.985	0.913-4.316	0..084
≥ 15%	25.7					
Family history						
No	59.6	1.532-6.805	0.002	1.963	0.867-4.448	0.106
Yes	44.4					

and capecitabine provided a clinical benefit rate at 42.1%-47.5% in metastatic Breast Cancer (BC) patients and a lower toxicity than capecitabine alone [26, 27] matching with our results in which the 4-year DFS was significantly higher for patients who received Cox-2 inhibitors (66% vs 41.9%) (p<0.001).

In another Phase II, multicentre, single-arm study (N001), concurrent celecoxib with neoadjuvant chemotherapy for 64 invasive BC patients achieved complete clinical response for 43 patients and partial clinical response for 13 [28].

In contrast to our results; long-term follow-up of the REMAGUS 02 trial, indicated that, in the addition of celecoxib as apart of neoadjuvant therapy for locally advanced TNBC subgroup led to lower T stage but no association of disease-free survival [29].

The 4-year DFS was significantly better for patients underwent MRM compared to conservative breast surgery (63.1% versus 38.3% respectively; p<0.001), while several controlled randomized trials demonstrated that overall survival of early-stage breast cancer cases treated with conservative breast surgery is nearly equal to modified radical mastectomy. But these trials did not explain the survival benefit for patients with TNBC treated with mastectomy or breast conservation with radiotherapy. In our study most of our patients received adjuvant radiotherapy even after mastectomy because most of our patients (68.6%) had lymph node involvement and >30% of cases represented with stage III [30, 31].

CONCLUSION

In conclusion, we reported that use of COX-2 inhibitors as maintenance therapy in patients with non-metastatic TNBC who have COX-2 expression of at least 10% associated with significant improvement in disease-free survival.

Our study was restricted to patients with non-metastatic TNBC who were treated and followed up at single university hospital. Our results should be assessed in multiple centres to include large patient numbers.

CONFLICT OF INTEREST

No conflict of interest was reported.

REFERENCES

1. Burstein MD, Tsimelzon A, Poage GM, Covington KR, Contreras A, et al: Comprehensive genomic analysis identifies novel subtypes and targets of triple-negative breast cancer. Clin Cancer Res. 2015;21:1688-1698.
2. Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res. 2007;13:4429-4434.
3. Lin NU, Claus E, Sohl J, Razzak AR, Arnaout A, et al. Sites of distant recurrence and clinical outcomes in patients with metastatic triple-negative breast cancer: high incidence of central nervous system metastases. Cancer. 2008;113:2638-2645.
4. Lin NU, Vanderplas A, Hughes ME, Theriault DO RL, Edge SB, et al. Clinicopathologic features, patterns of recurrence, and survival among women with triple-negative breast cancer in the national comprehensive cancer network. Cancer. 2012;118:5463-5472.
5. Smid M, Wang Y, Zhang Y, Sieuwerts AM, Yu J, et al. Subtypes of breast cancer show preferential site of relapse. Cancer Res. 2008;68:3108-3114.
6. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010;363:1938-1948.
7. Rampurwala M, Wisinski KB, O'Regan R. Role of the androgen receptor in triple-negative breast cancer. Clin Advanc Hematol Oncol. 2016;14:186-193.
8. Nakhjavani M, Hardingham JE, Palethorpe HM, Price TJ, Townsend AR. Druggable molecular targets for the treatment of triple negative breast cancer. J Breast Cancer. 2019;22:341-361.
9. Li Z, Qiu Y, Lu W, Jiang Y, Wang J. Immunotherapeutic interventions of triple negative breast cancer. J Transl Med. 2018;16:147.
10. Bocca C, Ievolella M, Autelli R, Motta M, Mosso L, et al. Expression of Cox-2 in human breast cancer cells as a critical determinant of epithelial-to-mesenchymal transition and invasiveness. Expert Opin Ther Targets. 2014;18:121-135.
11. Lucci A, Krishnamurthy S, Singh B, Bedrosian I, Meric-Bernstam F, et al. Cyclooxygenase-2 expression in primary breast cancer predicts dissemination of cancer cells to the bone marrow. Breast Cancer Res Treat. 2009;117:61-68.
12. Tian J, Hachim MY, Hachim IY, Dai M, Lo C, et al. Cyclooxygenase-2 regulates TGFβ-induced cancer stemness in triple-negative breast cancer. Sci Re. 2017;7:40258.
13. Witton CJ, Hawe SJ, Cooke TG, Bartlett JM. Cyclooxygenase 2 (COX2) expression is associated with poor outcome in ER-negative, but not ER-positive, breast cancer. Histopathol. 2004;45:47-54.
14. Yadav BS, Chanana P, Jhamb S. Biomarkers in triple negative breast cancer: A review. World J Clin Oncol. 2015;6:252-263.
15. Gelmon K, Dent R, Mackey JR, Laing K, McLeod D, et al. Targeting triple-negative breast cancer: optimizing therapeutic outcomes. Ann Oncol. 2012;23:2223-2234.
16. Miglietta A, Toselli M, Ravarino N, Vencia W, Chiecchio A, et al. COX-2 expression in human breast carcinomas: correlation with clinicopathological features and prognostic molecular markers. Expert Opin Ther Targets. 2010;14:655-664.
17. Kim HS, Moon HG, Han W, Yom CK, Kim WH, et al. Cox2 overexpression is a prognostic marker for stage III breast cancer. Breast Cancer Res Treat. 2012;132:51-59.
18. Zerkowski MP, Camp RL, Burtness BA, Rimm DL, Chung GG. Quantitative analysis of breast cancer tissue microarrays shows high cox-2 expression is associated with poor outcome. Cancer Invest. 2007;25:19-26.
19. Lund MJ, Trivers KF, Porter PL, Coates RJ, Leyland-Jones B, et al. Race and triple negative threats to breast cancer survival: a population-based study in Atlanta, GA. Breast Cancer Res Treat. 2009;113:357-370.
20. Telli ML, Gradishar WJ, Ward JH. NCCN Guidelines Updates: Breast Cancer. J Natl Compr Canc Netw. 2019;17:552-555.
21. Nakase T, Ueno M, Uchiyam K, Matsuur N, Yamaue H. Expression of cyclooxygenase-2 and transforming growth factor-beta 1 in patients with the early recurrence of hepatocellular carcinoma following hepatectomy. Surgical Sci. 2012;3:322-333.
22. John IPA, Jennifer WJ, Eric-Jan W, Uri S, Christopher CD, et al. A manifesto for reproducible science. Nature Human Behav. 2017;1:1-21.
23. Rosas C, Sinning M, Ferreira A, Fuenzalida M, Lemus D. Celecoxib decreases growth and angiogenesis and promotes apoptosis in a tumor cell line resistant to chemotherapy. Biol Res. 2014;47:1-27.
24. Bocca C, Bozzo F, Bassignana A, Miglietta A. Ant proliferative effects of COX-2 inhibitor celecoxib on human breast cancer cell lines. Mol cell Biochem. 2011;350:59-70.
25. Young SD, Lafrenie RM, Clemons MJ. Phase ii trial of a metronomic schedule of docetaxel and capecitabine with concurrent celecoxib in patients with prior anthracycline exposure for metastatic breast cancer. Curr Oncol. 2012;19:e75-e83.
26. Fabi A, Metro G, Papaldo P, Mottolese M, Melucci E, et al. Impact of celecoxib on capecitabine tolerability and activity in pretreated metastatic breast cancer: results of a phase II study with biomarker evaluation. Cancer Chemother Pharmacol. 2008;62:717-725.
27. Chow LW, Tung SY, Ng TY, Ty N, Im SA, et al. Concurrent celecoxib with 5-fluorouracil/epirubicin/cyclophosphamide followed by docetaxel for stages II-III invasive breast cancer: the OOTR-N001 study. Expert Opin Investig Drugs. 2013;22:299-307.
28. Giacchetti S, Hamy AS, Delaloge S, Brain E, Berger F. Long-term outcome of the REMAGUS 02 trial, a multicenter randomized phase II trial in locally advanced breast cancer patients treated with neoadjuvant chemotherapy

with or without celecoxib or trastuzumab according to HER2 status. Eur J Cancer. 2017;75:323-332.

29. Van Maaren MC, De Munck L, De Bock GH, Jobson JJ, van Dalen T, et al. 10 year survival after breast-conserving surgery plus radiotherapy compared with mastectomy in early breast cancer in the Netherlands: a population-based study. Lancet Oncol. 2016;17:1158-1170.

30. Adkins FC, Gonzalez-Angulo AM, Lei X, Hernandez-Aya LF, Mittendorf EA, et al. Triple-negative breast cancer is not a contraindication for breast conservation. Ann Surg Oncol. 2011;18:3164-3173.

31. Simone NL, Dan T, Shih J, Smith SL, Sciuto L, et al. Twenty-five year results of the national cancer institute randomized breast conservation trial. Breast Cancer Res Treat. 2012;132:197-203..