Biomarkers for evaluating response to chemotherapy in metastatic breast cancer patients

Nidhal A.K. Mohammed Ali * **BVMS, PhD Pharmacology**
Rehab M.Younis ** **BSc, MSc Pharmacology**
Jangi S. Salai** **BVMS, PhD Molecular Biology**

Fac Med Baghdad 2017; Vol.59, No.2
Received: Oct. 2016
Accepted: Jan .2017

Abstract:

Background: Although, different protocols of chemotherapy are recommended for the treatment of metastatic breast cancer, still response rates are variable.

Objectives: The aim of this study is to investigate the effects and correlation of different chemotherapy administered to metastatic breast cancer patients on serum levels of some biomarkers.

Patients and methods: Thirty metastatic breast cancer patients were enrolled in the study. The patients received different protocols of chemotherapy. Blood samples were taken from the patients before and after the last cycle of each protocol and from 20 healthy control and serum levels of biomarkers IL-6, leptin, CA 15-3 and p53 were estimated by Elisa.

Results: The mean serum levels of IL-6, leptin, CA 15-3 and p53 were significantly ($P \leq 0.01$) higher in patients before and after chemotherapy compared to controls. A significant ($P \leq 0.01$) higher mean levels of IL-6, leptin and p53 was found in the patients after chemotherapy compared to before chemotherapy with the exception of mean serum levels of CA 15-3 that did not differed significantly ($P \geq 0.174$) after chemotherapy compared to before chemotherapy. A significant correlation was found between hormone status and the mean serum level of leptin and between the mean serum levels of CA15-3 and IL-6.

Conclusion: The results highlights that the biomarkers IL-6, leptin, CA 15-3, p53 play a role in breast cancer progression and metastasis and could be helpful in predicting and monitoring chemosensitivity to these chemotherapeutic drugs.

Keywords: breast cancer, chemotherapy, IL-6, leptin, CA 15-3, p53.

Introduction:

Breast cancer is the second leading cause of death from cancer in women 1. The standard systemic treatments for metastatic breast cancer include cytotoxic chemotherapy, targeted therapy and endocrine therapy 2. Various advances have been achieved in the treatment of metastatic breast cancer, but prognosis is still deficient 3. Increasing incidence of treatment-resistant metastatic disease, and failure of chemotherapy in eliminating cancer stem cell (CSC) population, also involved in poor prognosis in those patients 4. Serum biomarkers may play important roles in the diagnosis, prognosis, predicting response to specific therapies, early detection of recurrence after curative surgery, understanding mechanisms of resistance 5. IL-6 is one of the most potent cytokine that has a major role in promoting tumor growth through inhibiting cancer cells apoptosis and inducing tumor angiogenesis6. CA 15-3, is widely used for monitoring therapy in patients with metastatic disease and elevated CA 15-3 levels found associated with poor response to chemotherapy and treatment failure 7. The p53 protein, facilitates antitumor drug response through a variety of key cellular functions, including cell cycle arrest, senescence, and apoptosis. However, these functions essentially cease once p53 become mutated 8.

**Dept of Pharmacology, College of Medicine, Hawler Medical University; Email: nidhal.mali@med.hmu.edu.krd.*
***College of Pharmacy, University of Mosul.*

In breast cancer, leptin which is secreted by adipose tissue is overexpressed and high leptin serum levels promote *in situ* estrogen production and directly trans activate estrogen receptor, increases expression of HER2 that promote invasion and resistance to therapy 9.

Patients and Methods:

Thirty female patients aged between 30-70 years with metastatic breast cancer who were attending Nanakaly ospital and Oncology Department in Rizgari Teaching Hospital, Kurdistan region, Erbil from December 2014 to September 2015 were enrolled in this study. Ethical committee approval from the College of Medicine and from both hospitals were obtained. The patients were instructed by the oncologist to receive chemotherapy in different protocols, 15 patients received Taxanes (Paclitaxel for 12 cycles and Docetaxel for 4 cycles), 8 patients AC (Adriamycin-Cyclophosphamide) and 7 patients Capecitabine for 4 cycles for each of them. A control group included twenty apparently healthy individuals with no history of cancer with age, BMI and sex matched with the patients group. Inclusions criteria were metastatic breast cancer (stage 4) or recurrence. Primary breast cancer, previous chemotherapy or hormonal therapy after diagnosis as metastatic breast cancer, and patients with liver, kidney impairment, and cardiac insufficiency were excluded in this study. Blood sample (5 ml) was withdrawn from each patient before administration of chemotherapy and after the last cycle of chemotherapy. Serum was separated and introduced

into small vacutainers and stored at -70°C for biomarkers assay IL-6, Leptin, CA15-3, and P53 ELISA. The biomarkers used were Leptin ELISA kit (Dbc, Canada), CA15-3 ELISA kit (Cobas, USA), Human IL-6 ELISA kit (AviBion, Finland), p53 ELISA kit (IBL, Germany).

Statistical analysis:

Data were analyzed statistically using SPSS Version 20.0 for Windows. All the data were expressed as Mean ± SD. Comparisons between groups was determined by Duncan test. Pearson correlation was used to find the correlation between different parameters. Mann-Whitney test was used to compare differences between two independent groups when the dependent variable is not normally distributed data. *P* value of 0.05 or less was considered statistically significant.

Results:

The age of patients ranged from 30-70 years with a mean of 48.8 ± 10.25 years and a mean body mass index (BMI) of 32.7 ± 8.74 (Table1). ER+ /PR+ was expressed in 66.7% patients and 33.3% patients expressed ER-/PR- while HER2+ve and HER2-ve was expressed in 40% and 60% of patients respectively and 10% patients expressed triple negative breast cancer (TNBC) as shown in table (1). Concerning chemotherapy; 50% of the patients received taxanes (paclitaxel and docetaxel), 26.7% received AC and 23.3% patients received capecitabine (Table 1). Patients with had metastasis to one site either bone, or liver or lung constituted 73.3% and 26.7% had metastasis to more than one site (Figure 1).

Table (1): Demographic characteristics of the patients (no=30) enrolled in the study

Age: Mean ± SD (years)	48.8 ± 10.25
BMI: Mean ± SD	32.7 ± 8.74
Hormonal Status	N (%)
ER+/PR+	20 (66.7%)
ER-/PR-	10 (33.3%)
HER2 status	**N (%)**
HER2+	12 (40%)
HER2-	18 (60%)
TN	3 (10%)
Metastatic site:	**N (%)**
1 metastatic site	22 (73.3%)
>1 metastatic site	8 (26.7%)
Chemotherapy protocol	**N (%)**
Taxanes	15 (50%)
Adriamycin-Cyclophosphamide (AC)	8 (26.7%)
Capecitabine	7 (23.3%)

Abbreviations: ER+, estrogen receptor; PR+, progesterone receptor; HER2, human epidermal growth factor receptor 2; TN, triple negative (ER-/PR-/HER2-)

A significant (P ≤ 0.01) higher mean serum level of IL-6 was found in the patients before and after administration of chemotherapy compared to control and between pre and post chemotherapy (Table 2).

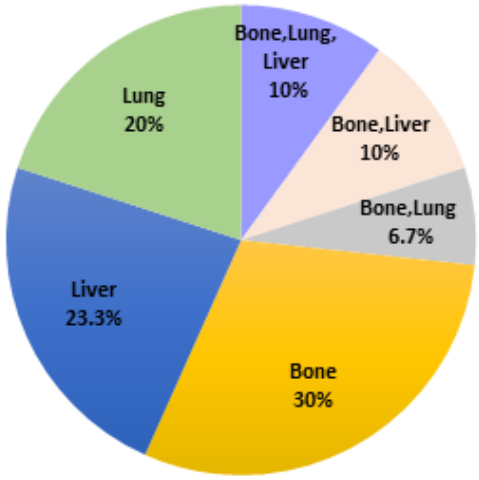

Figure (1): Distribution of patients according to metastatic sites.

The mean serum leptin level was significantly (P≤0.01) higher in patients before and after administration of chemotherapy compared to control subjects and also after receiving chemotherapy compared to those before receiving chemotherapy (Table 2). The mean level of CA15-3 in serum of patients after administration of chemotherapy was non-significantly (*P* ≥ 0.174) different from those before chemotherapy but both were significantly (P ≤ 0.01) higher than those of controls (Table 2). The mean p53 serum level increased significantly (P≤ 0.01) after receiving chemotherapy than pre-treatment level and also each was significantly higher than those of control (Table 2).

Table (2): Mean levels of IL-6, leptin, p53and CA15-3 in serum of control subjects and in metastatic breast cancer patients (n= 30) before and after administration of chemotherapy.

Biomarker	Healthy Control	Before Chemo-therapy	After Chemo-therapy	*P* Value
Il-6 (pg/ml)	3.83±0.18 ^{A}	9.87±2.28 ^{B}	22.65±3.97 ^{C}	0.0001
Leptin(ng/ml)	13.22±3.56 ^{A}	40.45±5.87 ^{B}	58.14±6.23 ^{C}	0.0001
CA15-3(U/ml)	17.42±1.16 ^{A}	148.36±29.68 ^{B}	125.92±29.40 ^{B}	0.174
P53(U/ml)	0.017±0.01 ^{A}	0.77±0.05 ^{B}	1.71±0.027 ^{C}	0.003

***P* value ≤ 0.05 was considered statistically significant. Different latter indicate significant differences between groups.**

No significant differences was found between mean serum levels of L-6, leptin, CA15-3 and p53, whereas, a significant difference (P ≤ 0.05) between serum level of leptin in patients with ER+/PR+ and ER-/PR- (Table 3).

Table (3): Mean serum levels of IL-6, Leptin, CA15-3 and p53 in HR+ patients and HR- patients (n= 30) after administration of chemotherapy.

Parameter	ER+/PR+ patients	ER-/PR- patients	P value
IL-6 (pg/ml)	26.22± 5.38	15.51 ± 4.63	0.209
leptin (ng/ml)	47.82± 6.59	78.79±11.00	0.016
CA15-3 (U/ml)	137.01±41.15	103.74±33.56	0.602
p53 (U/ml)	1.89 ± 0.39	1.34 ± 0.26	0.354

P value ≤ 0.05 was considered statistically significant

There was no significant differences in the mean serum IL-6, Leptin, CA15-3 and p53 levels in patients with 1 metastatic site and > 1 metastatic site patients (Table 4) and after administration of different chemotherapy protocols (Table 5). A statistical significant ($P \leq 0.039$) correlation ($r = 0.392$) was found only between IL-6 and CA15-3 serum level after administration of different chemotherapy protocols (Figure 2).

Table (4): Mean serum IL-6, Leptin, CA15-3 and p53 level in patients with 1 metastatic site and> 1 metastatic site patients (n= 30) after administration of different chemotherapy protocols.

Parameter	1 metastatic site patients	>1 metastatic site patients	P value
IL-6 (pg/ml)	19.32 ± 3.88	31.83 ± 10.2	0.167
leptin (ng/ml)	60.0 ± 7.18	53.04±13.16	0.630
CA15-3 (U/ml)	115.69±33.09	154.07±65.13	0.573
p53 (U/ml)	1.89 ± 0.35	1.2 ± 0.3	0.276

P value ≤ 0.05 was considered statistically significant

Table (5). Mean serum level of IL-6, Leptin, CA15-3 and p53, in patients (n= 30) after administration of taxanes, AC, and capecitabine

Parameter	Patients on Taxanes	Patients on AC	Patients on capecitabine	P value
IL-6 (pg/ml)	18.51±5.95	23.4± 5.83	30.69±9.28	0.485
Leptin (ng/ml)	53.31±7.0	58.59± 15.34	68±14.17	0.658
CA15-3 (U/ml)	93.73±36.72	159.8± 65.29	156.08±68.84	0.583
p53 (U/ml)	1.97±0.45	1.49± 0.55	1.39±0.3	0.641

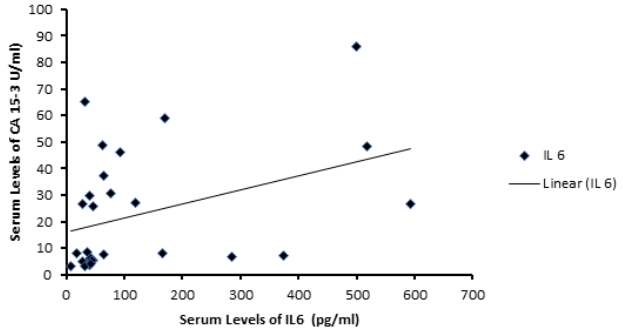

Figure (2): Correlation between serum levels of IL-6 and CA 15-3 in the metastatic breast cancer patients (n=30).

Discussion:

Despite improvements in treatment options, breast cancer remains one of the leading causes of mortality in women 10. The average BMI (32.7±8.74) of the patients in this study indicate the patients are overweight or obese according to the WHO recommended BMI cut-off points for overweight and obesity as ≥25 kg/m2 and ≥30 kg/m2 respectively 11. Evidences exist that breast cancer risk is positively associated with BMI in postmenopausal women and they have a 25% increase in the risk of developing breast cancer 12. In addition, obese women with breast cancer have more chance of treatment failure when compared to lean women 1. Endocrine changes that contributes to increased risk of developing breast cancer in obese postmenopausal women is related to increased activity of aromatase enzyme that results in increased formation of estradiol 13. The lack of significant ($P \geq 0.311$) correlation between mean p53 serum level and HER2 overexpression in this study has also been reported with taxanes as high expression of HER2 and p53 found associated with advanced stage of the cancer and with poor survival in breast cancer 14 and that HER2 signaling cascade influences activity of several chemotherapy by altering cell survival, apoptosis, drug efflux, and drug metabolism 14. The significantly ($P \leq 0.05$) higher serum IL-6 levels in the patients in the current study after receiving chemotherapy as compared to control and before administration of chemotherapy, has been demonstrated that increased IL-6 serum levels in tumor microenvironment protect cancer cells against chemotherapy, increases the expression of several survival proteins, including Bcl-2 and Bcl-xL and confers resistance to apoptosis induced by doxorubicin, paclitaxel, and trastuzumab and associate with worse survival in patients with metastatic breast cancer and correlate with the extent of disease migration 15,16,17. In breast cancer, doxorubicin found to induce inflammation, NF-κB, VEGF, and increased levels of inflammatory markers such as IL-1β and IL-6, promote bone and lung metastasis through inducing epithelial mesenchymal transition (EMT) mediated by production of TGF-β as well as tumor cell migration and invasion 18. Data showed that multidrug resistant cells have a stronger immune-suppressive attitude than chemosensitive cells, due to the constitutive activation of the JAK/STAT3/indoleamine 2, 3-dioxygenase 1 axis, thus resulting in chemoresistance to doxorubicin and paclitaxel 19. This evidence may also reflect the significantly ($P \leq 0.05$) higher serum IL-6 levels in the patients in this study after receiving chemotherapy as compared to those before chemotherapy. Numerous *in vitro* and *in vivo* studies reported that IL-6 is capable of modulating the expression and function of different drug transporters that increases their resistance to chemotherapeutic agents including doxorubicin, and paclitaxel 20. This fact might explain the significantly ($P \leq 0.0001$) higher IL-6 serum levels after administration of chemotherapy compared to serum levels before chemotherapy

assuming the tumor cells in the patients might had developed resistance to these chemotherapy. Other reason for the significantly higher serum IL-6 levels in the patients in the current study after receiving chemotherapy besides implication of resistance could be attributed to the role of cancer stem cells (CSC) which has been shown to increase resistance to radiation, chemotherapy such as docetaxel, trastuzumab, and tamoxifen 21. The mean serum levels of leptin that revealed a significant elevation ($P \leq 0.05$) in breast cancer patients before treatment compared to controls is in agreement with the findings of other studies that demonstrated also correlation of leptin with increased risk of breast cancer and associated with distant metastasis 22. Breast tumor cells found to acquire high ObR expression under the adiposity enriched environment and generate a population with enhanced CSC properties, tumorigenic capacity involved in the stabilization of CSC phenotype 23. The role of leptin in breast cancer is crucial as interplay in mediating tumor-stroma interaction and influencing EMT-linked mechanisms; which is an early step during tumor metastasis that sustain breast cancer growth and progression 24 ; this evidence, might explain the significantly ($P \leq 0.05$) higher levels of leptin in the patients in this study compared to controls. It has been reported that ERα plays an important role in chemoresistance to paclitaxel hindering the cytotoxic effects 25, this might explain the significantly ($P \leq 0.05$) higher serum levels of leptin in the patients after chemotherapy when compared with the levels before chemotherapy administration. Evidence has indicated that leptin and estrogen might cooperate in maintaining estrogen-dependent breast cancer growth 24. These statements might explain the significant ($P \leq 0.05$) correlation between leptin and HR+ shown in this study. Other data showed that leptin promote cellular proliferation not only in ER+ but also in ER- breast cancer cell lines (25). This evidence was based on a strong correlation between leptin signaling and expression of an oncogenic enzyme; Sphingosine kinase 1 (SK1) in breast tumors, which have a physiological significance in obesity driven ER- breast cancer 26. This finding most likely explain the significant correlation ($P \leq 0.05$) between leptin levels and HR- status in the patients in this study. There have been lack of reports investigating prognostic effects of leptin in metastatic breast cancer patients after chemotherapy that support the potential use of leptin-signaling inhibition as a novel treatment for ER+ and ER- breast cancer. The usefulness of measuring CA 15-3 levels in patients with breast cancer remains controversial 2. The significant ($p \leq 0.05$) higher mean serum levels of CA 15-3 in breast cancer patients pre and post treatment respectively compared to normal healthy controls indicate it is overexpressed during metastasis and is useful as a tumor marker for metastatic breast cancer patients follow up since elevation of CA 15-3 serum levels greater than the cutoff 30 U/ml shown correlated with tumor progression and malignancy 27. Monitoring CA 15-3 levels is recommended to follow up patients receiving chemotherapeutic or anti-hormonal therapy and increased CA 15-3 serum levels was considered a marker reflect signal of the presence of tumor and metastasis 7. Indeed in this study, the patients had metastasis to different sites (bones, liver, and lung) therefore, the significant elevation in CA 15-3 serum levels before chemotherapy compared to controls probably coincide with these observations. The significant ($P \leq 0.05$) correlation that was found between serum levels of CA 15-3 and IL6 in the patients after chemotherapy most likely express the insufficient control of cancer by the chemotherapy and reflect possibility of chemotherapy resistance since STAT3 activation is associated with tumor survival and therapeutic resistance along with pro-metastatic behavior reported for paclitaxel 28 and illustrate metastasis particularly 30% of the studied patients had bone metastasis. The mean p53 serum levels that was significantly ($P \leq 0.05$) higher in the patients in this study before and after receiving chemotherapy respectively as compared to controls indicate that this tumor suppresser protein is upregulated in these patients. Mutation of p53 have been described in breast cancer and observed in 30% of breast carcinomas 8 and found to contribute to tumorigenesis and confer aggressive tumor behavior that is not seen in p53-wild type 29. Under normal conditions, the wild-type p53 protein is normally undetectable or expressed at low levels in all human cells because it is rapidly degraded with short half-life (~20 minutes) except under conditions of cellular stress. In contrast, mutant proteins is often associated with the production of a stable protein, which increases the half-life that is readily detectable 29, 30. On this ground, in the present study, the mean serum level of p53 that was detected in the apparently healthy control was considered the cutoff value for normal p53, whereas; the levels in the patients before and after chemotherapy respectively are considered indicative for the mutant p53 31. A possible explanation for the significant ($P \leq 0.05$) higher mean serum levels of p53 in the patients after administration of chemotherapy compared to those before chemotherapy, could be related collectively to; p53 overexpression, or chemoresistance to taxanes, doxorubicin, and to tamoxifen; that is administered along with chemotherapy to the patients Since the correlations between mean serum levels of p53 in patients in the current study with HR+ and HR- was not significantly different, thus, it is most likely expected that these patients are resistant to the effect of administered chemotherapeutic agents. Thus, effective treatment for these tumors expressing ER, may require the concomitant complete inhibition of ER signaling with drugs such as fulvestrant, a drug that leads to the down-regulation and degradation of the ER together with treatments that promote p53-mediated apoptosis 32. Recent studies have demonstrated that p53 directly activate Toll-like receptor 4 (TLR4) and in p53 mutant

breast cancer cells, TLR4 activation alters the balance of progrowth and antigrowth cytokines in the extracellular microenvironment, ultimately resulting in increased proliferation and growth and promote chemoresistance with taxanes chemotherapy 33.34. Furthermore, paclitaxel shown to induce activation of TLR4-NF-κB pathway leading to upregulation of pro-survival genes Bcl-2, Bcl-xL proteins besides mutant p53, enhances the survival of cancer cells promote chronic inflammation, peripheral neuropathy, angiogenesis, and recovery of damaged cells 34,35. Collectively, these results demonstrate that paclitaxel not only kills tumor cells but meanwhile it enhances their survival by activating TLR4 pathway. Therefore; a possible explanation for the significant ($P \leq 0.05$) increase in p53 levels shown in patients in this study after chemotherapy compared to control apparently healthy and to those before receiving chemotherapy is most likely attributed to either; the increase in mutant p53 levels as consequence to chemotherapy induced activation of TLR4 by the taxanes and doxorubicin that subsequently leads to activation of transcription factor NF-κB, a prosurvival factor and causes upregulation of p53 or to chemoresistance induced by MDR and metastasis 36.The ability of paclitaxel in increasing tumor cell invasion is of particular significance and a variety of human cancer cell lines, showed that paclitaxel is one of the most potent inducers of invadopodia, which is a cell protrusion required for invasion 37. Besides these, hypoxia also shown to induce mutant p53 as well as resistance to paclitaxel through reactive oxidative species (ROS)-mediated HIF-1α stabilization 38. The central role of p53 in the control of apoptosis, senescence and cell cycle arrest, remains a puzzle for its possible role in predicting response to chemotherapy in breast cancer patients as there are unconvincing response for tumors with functional or mutant p53. Attempts are required to be directed toward personalized medical treatment because of genomic variation including polymorphisms, gene expression levels, and epigenetic changes in both the host and tumor tissue and another point that should not be forgotten is to weigh the benefits over the risk of cancer 39, 40. The non-significant differences in the mean serum levels of the biomarkers, in patients with 1 metastatic site and > 1 metastatic site and after administration of different chemotherapy protocols obviously demonstrate insufficient control by different chemotherapy in the studied patients.

Conclusion:

There remain gaps in our knowledge that demand further research, for understanding the factors contributing to cancer occurrence, metastasis, and drug resistance. Therefore, investigating the potential role of tumor markers is of high importance to fill the gaps between basic research and clinical application.

Authors Contributions:

Nidhal AK Mohammed Ali: Research designer, arrangement and follow up collection, analysis of samples and data and writing the manuscript.

Rehab Mohammed Younis: Collecting samples, analysis of samples and writing the manuscript.

Jangi S. Salai: Facilitate collection of blood samples in the hospital

References:

1- *Ferlay J, Soerjomataram I, Dikshit R, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN. Int J Cancer. 2015; 136: 359–86.*

2- *National Comprehensive Cancer Network [Web page]. National Comprehensive Cancer Network (NCCN) clinical practice guidelines in oncology, breast cancer. Version 1. Available at: http://www.nccn.org/professionals/physician_gls/pdf/breast.pdf; 2011 [cited April 10 2013].*

3- *Roche H, Vahdat LT. Treatment of metastatic breast cancer: second line and beyond. Annals of Oncol. 2012; 22: 1000–10.*

4- *Yapeng Hu, Liwu Fu. Targeting cancer stem cells: a new therapy to cure cancer patients. Am J Cancer Res. 2012; 2 (3): 340-56.*

5- *Guo H, Zhou X, Lu Y, et al. Translational progress on tumor biomarkers. Thorac. Cancer. 2015; 6 (2015): 665–71.*

6- *Chang Q, Bournazou E, Sansone P, et al. The IL-6/JAK/Stat3 feed-forward loop drives tumorigenesis and metastasis. Neoplasia. 2013; 15 (7): 848-62.*

7- *Wu SG, He ZY, Ren HY, et al. Use of CEA and CA15-3 to Predict Axillary Lymph Node Metastasis in Patients with Breast Cancer. J Cancer. 2016; 7 (1): 37-41.*

8- *Bertheau P, Lehmann-Che J, Varna M, et al. P53 in breast cancer subtypes and new insights into response to chemotherapy. Breast. 2013; 22 (S2): 27-29.*

9- *Giordanoa C, Vizzab D, Panza S, et al. Leptin increases HER2 protein levels through a STAT3-mediated up-regulation of Hsp90 in breast cancer cells. Mol. Oncol. 2013; 7: 379 -391.et al. Kyriakides S, Senkus E; ESMO Guidelines Working Group. Locally recurrence or metastatic breast cancer: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2012; 23 (S7): 11-19.*

10- *Reeves GK, Pirie K, Beral V, et al. Cancer incidence and mortality in relation to body mass index in the Million Women Study: cohort study. BMJ. 2007; 335 (7630): 1134-45.*

11- *Widschwendter P, Friedl TW, Schwentner L, et al. The influence of obesity on survival in early, high-risk breast cancer: results from the randomized SUCCESS A trial. Breast Cancer Res.2015; 17:129-40.*

12- *Fontanella C, Lederer B, Gade S, et al. Impact of body mass index on neoadjuvant treatment outcome: a pooled analysis of eight prospective neoadjuvant breast cancer trials. Breast Cancer Res Treat. 2015; 150 (1): 127-39.*

13- *Rose DP, Vona-Davis L. Interaction between menopausal status and obesity in affecting breast cancer risk. Maturitas. 2010; 66 (1): 33-8.*
14- *Camerini A., Donati S., Viacava P., et al. Evaluation of HER2 and p53 expression in Predicting response to docetaxel-based first-line chemotherapy in advanced breast cancer. Journal of Experimental & Clinical Cancer Research 2011; 30: 38- 47.*
15- *Ara T and Declerck YA. Interleukin-6 in Bone Metastasis and Cancer Progression. Eur J Cancer.2010; 46 (7): 1223–1231.*
16- *Korkaya H, Kim GI, Davis A, et al. Activation of an IL6 inflammatory loop mediates trastuzumab resistance in HER2+ breast cancer by expanding the cancer stem cell population. Mol Cell. 2012; 47 (4): 570-84.*
17- *Oh K, Lee OY, Shon SY, et al. A mutual activation loop between breast cancer cells and myeloid-derived suppressor cells facilitates spontaneous metastasis through IL-6 trans-signaling in a murine model. Breast Cancer Res. 2013; 15 (5): 79-85.*
18- *Vyas D, Deshpande K, Chaturvedi L, et al. Rapid Extensive Recurrence of Triple Negative Breast Cancer: Are Both Therapy and Cancer Biology the Culprit. J Clin Med Res. 2016; 8 (2): 162-67.*
19- *Campia I, Buondonno I, Castella B, et al. An Autocrine Cytokine/JAK/STAT-Signaling Induces Kynurenine Synthesis in Multidrug Resistant Human Cancer Cells. PLoS One. 2015; 10 (5): 126159- 69.*
20- *Guo Y, Xu F, Lu T, et al. Interleukin-6 signaling pathway in targeted therapy for cancer. Cancer Treat Rev.2012; 38 (7): 904–10.*
21- *Maycotte P, Jones KL, Goodall ML, et al. Autophagy Supports Breast Cancer Stem Cell Maintenance by Regulating IL6 Secretion. Mol Cancer Res. 2015; 13 (4): 651-58.*
22- *Chang CC, Wu MJ, Yang JY, et al. Leptin–STAT3–G9a Signaling Promotes Obesity-Mediated Breast Cancer Progression. Cancer Res. 2015; 75 (11): 1–12.*
23- *Wolfson B, Eades G, Zhou Q. Adipocyte activation of cancer stem cell signaling in breast cancer. World J Biol Chem. 2015; 6 (2): 39-47.*
24- *Barone I, Catalano S, Gelsomino L, et al. Leptin mediates tumor-stromal interactions that promote the invasive growth of breast cancer cells. Cancer Res. 2012; 72: 1416–27.*
25- *Jiang Z, Guo J, Shen J, et al. The role of estrogen receptor alpha in mediating chemoresistance in breast cancer cells. J Exp Clin Cancer Res. 2012; 31: 42-52.*
26- *Alshaker H, Krell J, Frampton AE, et al. Leptin induces upregulation of sphingosine kinase 1 in oestrogen receptor-negative breast cancer via Src family kinase-mediated, janus kinase 2-independent pathway. Breast Cancer Res. 2014; 16: 426- 31.*
27- *Geng B, Liang MM, Ye XB, et al. Association of CA 15-3 and CEA with clinicopathological parameters in patients with metastatic breast cancer. Mol Clin Oncol. 2015; 3: 232-36.*
28- *Richard L. Carpenter 1, Hui-Wen L. STAT3 Target Genes Relevant to Human Cancers. Cancers 2014; 6: 897-925.*
29- *Parrales A, Iwakuma T. Targeting Oncogenic Mutant p53 for Cancer Therapy. Front Oncol. 2015; 5: 288- 91.*
30- *Vijayakumaran R, Tan KH, Miranda PJ, et al. Regulation of Mutant p53 Protein Expression. Front Oncol.2015; 5: 284-88.*
31- *Burgess A, Chia KM, Haupt S, et al. Clinical Overview of MDM2/X-Targeted Therapies. Front Oncol.2016; 6 (7): 1-7.*
32- *Fernandez-Cuesta L, Anaganti S, Hainaut P, et al. P53 status influences response to tamoxifen but not to fulvestrant in breast cancer cell lines. Int. J. Cancer. 2010; 128 (8): 1813–21.*
33- *Haricharan S, Brown P. TLR4 has a TP53-dependent dual role in regulating breast cancer cell growth. Proc Natl Acad Sci USA. 2015; 112 (25): 3216-25.*
34- *Ran S. The Role of TLR4 in Chemotherapy-Driven Metastasis. Cancer Res. 2015; 75 (12): 2405-10.*
35- *Volk-Draper L, Hall K, Griggs C, et al. Paclitaxel therapy promotes breast cancer metastasis in a TLR4-dependent manner. Cancer Res.2014; 74(19): 5421-34.*
36- *Xu F, Wang F, Yang T, et al. Differential drug resistance acquisition to doxorubicin and paclitaxel in breast cancer cells. Cancer Cell Int. 2014; 14: 142-55.*
37-*Quintavalle M, Elia L, Price JH, et al. A cell based high-content screening assay reveals activators and inhibitors of cancer cell invasion. Sci Signal. 2012; 4 (183): 49-62.*
38-*Yun ZH, Glazer PM. Tumor suppressor p53 stole the AKT in hypoxia. J Clin Invest. 2015; 125 (6): 2264–66.*
39-*Goldhirsch A, Wood WC, Coates AS, et al. Strategies for subtypes-dealing with the diversity of breast cancer: highlights of the St Gallen international expert consensus on the primary therapy of early breast cancer. Ann. Oncol. 2011; 18 (7): 1133-44.*
40-*Koleck TA, Conley YP. Identification and prioritization of candidate genes for symptom variability in breast cancer survivors based on disease characteristics at the cellular level. Breast Cancer. 2016; 8: 29–37.*