

## Antioxidant Modulation of Hematological Toxicity during Chemotherapy for Breast Cancer

Hojouj Mohammad\*, Bondarenko I, Zavizion V, Artemnko M, Soloviova N, Bondarenko Y, Dmytrenko K and Urzhumova N

Department of Oncology and Medical Radiology, Dnepropetrovsk Medical Academy, 31, Blyzhnya Str, Dnepropetrovsk, 49102, Ukraine

### Abstract

Side effects of anticancer drugs seriously limit the achievement of the maximum therapeutic effect of the most cytostatic. One of the pathophysiological bases of side effects is the ability of cytotoxic agents to intensify the free-radical processes and the consequent lipid peroxidation (LPO) in the cell membranes of various organs. When the antitumor treatment is conducted, the antioxidant enzyme deficiency increases, there is the depletion of non-enzymatic and enzymatic mechanisms of oxidation protection units, resulting in a reduction of organism resistance and damage to vital organs and systems. In this connection it is important to study the possibility of correction of violations occurring in cancer patients with drugs with antioxidant action type.

**Keywords:** Cytostatic; Molecular endocrine therapy; Oxidative stress; Tumor cells; Immunohistochemical

### Introduction

#### Objectives of the Study

1. To investigate the condition of antioxidant system in the blood of oncological patients during chemotherapy for breast cancer;
2. To study the efficacy of antioxidant therapy in the prevention of the development of leukopenia, as well as the possibility of improving treatment outcomes in their use;
3. To justify the use of antioxidant therapy for the prevention of hematological toxicity of chemotherapy according to levels of biochemical markers of the connected systems of antioxidant protection and oxidative stress.

### Case Study

#### Individualize treatment of hormone-dependent breast cancer

Around 13 500 female is diagnosed with breast cancer in Ukraine. System control in both local and recurrent breast cancer is topical issue and should be thoroughly discussed considering economic situation in country.

Standard drug treatment regimen does not always give positive results. Practically speaking, physicians use standard treatment regimen based upon tumor IHC analysis, if any.

Immunohistochemical study of breast cancer biological markers has two endpoints: 1) identify high risk groups for progression in early stage disease; additional examination and Treatment is required; 2) evaluation of individual responsiveness to planned or received treatment. Surrogate molecular classification is used to identify luminal subtypes, Her2-positive, basal and rare types. Luminal subtypes includes subtype A, about 40% of all breast cancer, this subtype characterized by positive estrogens (ER), progesterone (PR), absence of growth factor hyper fixation (Her2/neu) and low level of Ki67 proliferation (<20%). Luminal subtype B is subdivided into Her2-positive and Her2-negative subtypes. Luminal Her2-negative subtype differs from luminal A subtype by higher or high level of Ki67 marker ( $\geq 20\%$ ). Luminal B Her2-positive subtype expresses Her/neu, and has high level of Ki67 marker. Cases where Her2/neu has low level of proliferation (Ki67<20%) should be analyzed separately [1-7].

Classic example of breast cancer individualization treatment is by determining of reproductive hormones and epidermal growth factor [8]. Prognostic value of ER and PR determination for endocrine therapy is confirmed by meta-analysis of 55 randomized trials with 37000 breast cancer patients. It is proved that ER expression in breast tumor indicates potential responsiveness to drug treatment, this is aimed to remove estrogens source and its effect recovering. PR is the first step in cell reaction to progestines and it determines cell responsiveness to some drugs, but mainly because it synthesis in breast cancer cells is prompt by estrogens. Presence of PR confirms ER functional activity. Breast cancer patients who has both or at least one of steroid hormones receptors have more favorable prognosis than those has no receptors. Thus 75% of patients with both positive receptors responses effectively to endocrine therapy and 50% with only ER positive receptor. However there was response to endocrine therapy in 10% of patient with both negative receptors [9].

A high level of estrogen receptors is associated, in front of all, with an increase in overall and not relapse-free survival, and is a predictor of the effectiveness of hormone therapy. In recent years Ki-67 proliferation index has been used to predict the effectiveness of treatment [10]. The panel of estrogen progestin receptor markers, Her2/neu and Ki-67, is today standard both at the stage of primary diagnosis and in morphological studies in the course of treatment. Taking into account these four markers, the main immunomorphologic subtypes of breast cancer are listed above. In the case of neoadjuvant treatment or treatment of metastatic cancer (if there is a possibility of re-biopsy in the dynamics), Ki-67 has recently been used as a marker for the effectiveness of treatment. Repeated biopsy is performed after 3 weeks of treatment. Decrease of marker level is the first morphological

**\*Corresponding author:** Mohammad H, Associate Professor Department of Oncology and Medical Radiology, Dnepropetrovsk Medical Academy, 31, Blyzhnya Str., Dnepropetrovsk, 49102, Ukraine, Tel: +380567560399/+380675673277; Fax: 380563777797; E-mail: [hojouj@yahoo.com](mailto:hojouj@yahoo.com)

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predictor of treatment efficacy, not only of hormone, but also of chemotherapy. Conversely, an increase in marker level is a predictor of an unfavorable forecast [11-15].

So, consider the information regarding the most favorable subtype - luminal A, and more aggressive, but still hormone-dependent - luminal B.

As long experience shows, the highest effectiveness of anti-estrogen therapy is observed, provided that the tumor expresses both estrogen receptors and progesterone receptors. The most commonly used drugs for anti-estrogen therapy are tamoxifen and toremifene. Preparations of choice include aromatase inhibitors (non-steroidal letrozole, anastrozole and steroid exemestan) and a selective inactivates of estrogen fulvestrant receptors. However, 10-15 percent of ER-positive tumors do not respond to tamoxifen, on the contrary, tumor growth may be noted [16-18].

The adverse effect of tamoxifen on the progression of breast cancer is strongly correlated with the amplification of the cyclin D1 oncogene. Blocking cyclones helps to eliminate proliferation, caused by estrogens and reduces the activity of estrogen receptors. Expression of cyclins activates extra genomic action of estrogens through stimulation of tyrosine kinase receptors or a self-assembly of functioning protein complexes in the cell's cytoplasm. These complexes include in the cascade other signaling molecules that activate transcription. Thus, with high expression of cyclin D1, anti-estrogen blocking of estrogen receptors will be less effective and the proliferation of tumor cells will be either due to the agonistic effects of tamoxifen or estrogen action of estrogens. Patients who have an enlarged copy of this gene should receive aromatase inhibitors as hormones, or abstain from the use of antagonists of the estrogen signaling cascade [19].

It should be taken into account that the negative or low level (up to 30%) of the expression of cyclin D1 is such as to promote tamoxifen. If the level of expression of cyclin D1 is medium to high, the action of tamoxifen is either agonistic or lost and triggers the mechanism of estrogen extracorporeal action. Thus, according to Skvortsov and Manichas Patients with a negative or low expression level of cyclin D1 had no recurrence of breast cancer during the five years of adjuvant tamoxifen, and in most patients with moderate to high expression, progression was recorded, mainly bone metastases (62% of patients with average level of expression and in 68% - with high). Defeat of soft tissues in the postoperative scar area occurred in 14% of patients with mean expression level and 28% with high expression of cyclin D1. Metastases in the lungs and 4% with high levels of soft tissue metastases occurred in 4% of patients with average levels of expression. Considering that even in advanced and super-radical operations, 70-80% of breast cancer patients who have not undergone additional treatment progress sooner or later [18], it is not difficult to conclude that the average and high levels of expression Cyclin D1 presumably causes tumor resistance to selective estrogen receptor modulators [20-22].

One of the markers of sensitivity to hormone therapy, some authors consider the regulator of apoptosis Bcl-2. Having studied the experience of many researchers, Baryshnikov and Stepanova argues that Bcl-2 can predict the efficacy of adjuvant therapy with tamoxifen in patients with a hormone susceptible breast cancer from damage to regional lymph nodes, especially in the group of ER negative tumors [20].

Other factors influencing the effectiveness of antagonists of estrogens are the estrogen receptor co-activator AIB1 and Her2/neu. The estrogen receptor co-activator AIB1 (amplified in breast 1) is also referred to as SRC-3 (steroid receptor coactivator-3), NCOA3 (nuclear

receptor coactivator 3) RFC3 (Replication factor C subunit 3). AIB1, like the estrogen receptor itself, is phosphorylated and thus functionally activates MARK (mitogen-activated protein kinase); thus, high levels of activated AIB1 may reduce the antagonistic effects of tamoxifen, especially in tumors that also overexpress HER/2. Thus, the expression of AIB1 is an important prognostic marker indicative of tamoxifen resistance. In this case, tumors expressing AIB1 remain susceptible to aromatase inhibitors [23,24].

Sensitivity data for Her2/neu positive tumors to tamoxifen are ambiguous. Herx/neu-expressing tumors are more likely to exhibit tamoxifen resistance than aromatase inhibitors. Thus, in study 024, "Letrozole versus tamoxifen in neoadjuvant breast cancer therapy", the objective response of c-crbB-positive tumors to letrozole therapy was in 88% of patients, while for tamoxifen alone, 21% of patients ( $p=0.0004$ ) [25]. The high effectiveness of aromatase inhibitors is also reported by Li Zhu. The analysis of the key findings of multicenter international clinical studies suggests the anticipated resistance of Her2/neu positive tumors to tamoxifen, but not to aromatase inhibitors. Later, the correctness of these findings was confirmed by clinical trial BIG 1-98 (80,010 patients were trailed). As one of the possible mechanisms for the development of estrogen receptor resistance of positive Her2/neu positive tumors, an adaptive increase in Her2/neu levels in response to long-term endocrine effects is considered. Thus, on the model of breast cancer cells with enormous resistance due to prolonged exposure to tamoxifen, it has been shown that the use of a double inhibitor of the tyrosine kinase receptor of the epidermal growth factor (lapatinib) leads to a restoration of sensitivity to tamoxifen. This effect is related to the genomic mechanism of action of estrogen receptors due to inhibition of mutual activation of EP and Her2. In any case, Her2/neu positive tumors are mostly tamoxifen resistant. However, they are quite often susceptible to aromatase inhibitors, and the addition of trastuzumab or lapatinib to therapy results in a significant improvement in the treatment outcome in patients with luminal Her2/neu positive breast cancer.

Another marker of tamoxifen resistance to hormone-dependent breast cancer is the mutation of the p53 gene. A mutation occurs in 10-20% of cases of luminal A and 13 to 31% of luminal B cell cancer [26] and is a marker of an unfavorable prognosis. Initially, the tamoxifen resistance of p53-mutated cells was shown in vitro on MCF-7 cell lines of breast cancer [27]. Subsequently, these data were confirmed in the meta-analysis of 4683 patients receiving tamoxifen after adjuvant chemotherapy.

The mechanism of resistance to aromatase inhibitors remains poorly understood. One of the probable mechanisms of acquired resistance to this group of drugs is the ectopic overexpression of aromatase by cancer cells. Another hypothesis - as a result of the long term absent estrogen aromatase inhibitors simulated the effect of the blockade of estrogen synthesis This mechanism of resistance is implemented through the PI3K/Akt/mTOR signaling pathway, To overcome the acquired resistance allows the use of mTOR inhibitors, the effectiveness of which is proven by clinical studies TAMRAD (tamoxifen+avorelimus), low level of progestin receptors, and their loss in the treatment process (regardless of the level of estrogen receptors) indicates a questionable sensitivity to inhibitors a romatasi [28].

In practice, the probability of such a mechanism of primary resistance is indicated by the presence of a metabolic syndrome in the patient, manifested before all in the increase in body weight ( $BMI > 25 \text{ kg/m}^2$ ) with the tendency or development of obesity before all by the abdominal type. The use of metformin (dimethylbiguanide)

in such patients helps to avoid the effect of primary resistance to aromatase inhibitors. Little is known about the molecular targets of the proapoptotic action of COX-2 inhibitors, although in a number of cases in apoptotic cells a violation of phosphorylation of kinase Akt (Protein kinase B alpha) has been noted [29].

- The HIF-1 $\alpha$ /VEGF signaling pathway (induced by hypoxia factor 1) plays an important role in the development of resistance to aromatase inhibitors. There is a direct correlation between the expression of HIF-1 $\alpha$  tumor cells and the resistance to neoadjuvant therapy with aromatase inhibitor (letrozole). Works that have a direct evaluation of HIF-1 $\alpha$  are few.

- The key target of HIF-1 $\alpha$  - vascular endothelial growth factor (VEGF) has been studied well. Estradiol stimulates the synthesis of VEGF, and the action of HIF-1 $\alpha$  and ER $\alpha$  in relation to VEGF is synergistic.

- The development of hormone resistance in breast cancer is accompanied by activation of the VEGF/VEGFR2/MAPK signaling pathway.

- Celecoxib (a specific VEGF inhibitor) affects the activity of the VEGF promoter and directly inhibits the synthesis of VEGF, in particular avoiding unwanted effects of tamoxifen

Thus, in the case of breast cancer diagnosis with the EP+PR+Her2/neu+++ phenotype, it is mandatory to study all markers Ki67, cyclin D1, AI.

The chemo-sensitivity of the tumor is appreciably correlated with the expression characteristics of certain molecules. Currently, molecular markers of fluoropyrimidine treatment efficacy are most studied: DPD, TS, TP TS (thymidylatesintetasa), a key phosphate-dependent mimetic enzyme in the process of DNA synthesis that catalyzes the formation of thymidylate, a precursor of thymidine triphosphate, a nucleotide necessary for the synthesis of DNA - and is the main target for the effect of uracil and 5 fluorouracil (5 active urethyl antimetabolites). The active metabolite of 5FU, 5-fluoro-2-deoxyuridine monophosphate (FdUMP), interacts with TS, resulting in blockage of DNA synthesis. The excess of the TSF 5F target leads to the fact that the transferable doses of the drug cannot "saturate" this enzyme and prove to be inevitably ineffective [30,31].

## Materials and Methods

The study of hematological parameters was evaluated in two groups of patients - control group (60 patients) and the study group (50 patients) with study drug. Both groups of patients received chemotherapy for breast cancer with taxanes+anthracyclines. In the period between courses of study group patients received antioxidant therapy. We studied the incidence of leucopenia during treatment. Also markers of protein oxidative modification, system of nitrogen oxide, thiol system and apoptotic markers were studied.

## Results and Discussion

1) After prophylactic administration of antioxidant drugs in the study group it was recorded a 2.05-fold greater level of thylol groups than in the control group of patients ( $p < 0.05$ ). In the study of spontaneous and stimulated markers of oxidative modification of the protein as a marker of oxidative stress, it was revealed that antioxidant therapy is effective protector of cell membranes from oxidative stress. Patient who received antioxidant drugs have 1, 27-77 times lower level of enzymes adelhydfenihydrazon and ketonefenihydrazon comparing

to the control group [32-35].

2) In the study group of patients who received antioxidant therapy, lower incidence of leukopenia (66.7% vs. 46.0%) ( $p < 0.05$ ) by reducing the incidence of deep leucopenia was observed. Indicators of anemia and thrombocytopenia occurring were not statistically different.

3) Reduction in the activity of antioxidant enzymes of glutathione level and the displacement of the thiol-disulfide equilibrium in the direction of reducing of the content of reduced forms indicates a reliable clinical need for the personal use of drugs with glutathione-protective effect and antioxidant potential.

## Conclusion

According to the results of a randomized clinical trial of PACS01, the addition of docetaxel to epirubicin and 5-fluorouracil as adjuvant chemotherapy in a group of patients with estrogen-positive tumors and a high Ki-67 index is advisable. These results were confirmed in the Cancer International Research Group 001 trial. However, these findings are not consistent with International Breast Cancer Study Group Trials VIII and IX. In these studies, the predictive role of the high level of Ki-67 expression in the group with receptor-positive breast cancer without signs of disease in the lymph nodes in relation to adjuvant therapy with the inclusion of methotrexate, cyclophosphamide and 5-fluorouracil in addition to endocrine therapy was demonstrated. Thus, it is important to conduct studies aimed at isolating groups of patients with high Ki-67 rates who can maximize the benefit of various adjuvant chemotherapy regimens.

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