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The Results of MSC-428 Nanomolecules Application in Neoadjuvant Therapy in Patients with Breast Cancer

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Abstract

Breast cancer takes the first place in the structure of morbidity and mortality from malignant neoplasms in women. It indicates the problem of patients and doctors' unsatisfied needs in the treatment of this disease. Some types of cancer, such as melanoma, bladder or renal cell carcinoma, demonstrated a long-term response to immunotherapy, however, breast tumors did not show the same efficacy, because breast cancer is immunologically "cold", it is weakly infiltrated with T cells and causes a weak immune response.

The causes of immune silence in breast cancer are not fully defined and this knowledge gap threatens the life and health of hundreds and thousands of patients.

Thus, further studies of new therapeutic agents, mixed agonists/antagonists of nanomolecules MSC-428 in multitarget immunotherapy targeted at reprogramming the activity of 8 receptor proteins, are crucial for the development of the most effective cancer treatment strategies.

According to the study, it can be stated that MSC-428 application in the combination with neoadjuvant chemotherapy (NAC) demonstrated the greatest clinical benefits for cancer patients, by involving the immune system in the process of tumor destruction. It made the tumor immunologically "hot", by enhancing its infiltration with T cells, reducing the risk of metastasis, inhibiting several very important immune checkpoint proteins, thereby increasing the cytotoxic effect of T-lymphocytes and, accordingly, the duration of disease-free survival of patients.

Keywords: Nanomolecules; Chemotherapy; Immunotherapy; Breast cancer

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Introduction

Breast cancer takes the first place in the structure of morbidity and mortality from malignant neoplasms in women. However, almost half (40–50%) of breast cancer patients first apply to the doctor having already stage III disease.

The main treatment strategy is neoadjuvant chemotherapy (NAC), but there are concerns about increasing resistance to it. There is evidence that chemotherapy can activate metastasis growth, neoangiogenesis, and further tumor growth [1].

In addition, the tumors themselves develop a multitude of mechanisms for evading immunity in the process of their development [2]. Besides, some types of cancer are inherently better "hidden" than the others. Due to a deeper understanding of the immunological surveillance of tumors, immunotherapy has become a promising strategy for treating breast cancer, despite the fact that historically it has been considered an immunologically silent tumor.

Until now, the causes of immune silence in breast cancer have not been fully defined. Thus, further research in cancer immunotherapy needs more thorough investigation to develop better strategies of treatment.

Following this aim, a study of 60 patients with locally advanced breast cancer was conducted to examine the effectiveness of the immunotherapeutic effect of innovative MSC-428 nanomolecules, which are applied simultaneously with neoadjuvant chemotherapy in breast cancer.

According to the conducted study, it can be stated that MSC-428 application in combination with neoadjuvant chemotherapy improves the results of antitumor therapy, allows to make the tumor immunologically "hot" by increasing its infiltration with T cells that makes neoadjuvant chemotherapy particularly effective [3].

In our opinion, the combined application of multitarget immunotherapy with innovative MSC-428 nanomolecules, targeted at several target proteins, predetermines a more effective treatment [4].

The combination immunotherapy/chemotherapy has a promising potential for better cancer treatment by involving the immune system in the process of tumor destruction.

Breast cancer is one of the most common tumor types, and metastasis greatly increases the risk of death from this disease [5,6]. By studying the process of intravasation or entry of cells into the vasculature, Karagiannis et al. discovered that, in addition to killing tumor cells, chemotherapy treatment can also increase intravasation. Groups of cells collectively known as tumor microenvironment of metastasis (TMEM) can serve as gateways for tumor cells entering the vasculature.

Accordingly, a new immunotherapeutic approach, which is able to potentiate the therapeutic effect of neoadjuvant chemotherapy, reduce the risk of metastasis and increase survival rate, is required [7,8]. In the available scientific resources we have not found any studies examining the dynamics of the expression of 8 protein receptors on the surface of lymphocytes plus phagocytosis systems in immunotherapy of breast cancer.

The absence of these data does not allow assessing in a proper way the activation or passivity of the proteins responsible for the cytotoxic potential of lymphocytes, the ability to infiltrate a tumor, to produce perforins and granzymes, etc.

As new MSC-428 nanomolecules possess the properties of mixed agonists / antagonists with respect to proteins CD3, CD4, CD8, CD16, CD25, CD38, CD45, CD95, it is absolutely logical to investigate the dynamics of change of these biomarkers that predetermines the understanding of the mechanisms of antitumor response in breast cancer.

Thus, this methodology has advantages over the others in such a way that it gives an opportunity to study the formation of the "correct algorithm" of the immune cells activity through 8 proteins (determined by CD) plus phagocytosis, that leads to the tumor destruction.

It is of current interest for the development of digital imaging of the pathological process modeling. Development of direction providing virtual pathology services at remote sites and facilitating consultations and second opinions. Immunotherapy has revolutionized cancer treatment. But despite this, there is still a low percentage of patients (18-20%) who respond to it. In our opinion, it is determined by narrow targeting effects of brand drugs on the cells of the immune system. Orientation at 1 or 2 target proteins predetermines limited efficiency. The object of the targeted effect should be a wider range of proteins. The methodology for studying the dynamics of changes in the activity of a larger number of lymphocyte receptor proteins provides a new vision of the multitarget immunotherapy opportunities [9,10].

Although the immune checkpoint inhibitors have been used in cancer immunotherapy in recent years, and their application in malignant tumors opens up a new page in the fight against cancer, the future of immunotherapy is in the identification of new biomarkers. As the role of immunotherapy in the first-line treatment and second-line treatment continues growing, the need for a more personalized and targeted approach based on lymphocyte biomarkers also increases.

The study of the therapeutic response, the duration of disease-free survival of patients and its correlation with the expression of lymphocyte markers (CD), makes an important contribution both to the existing scientific literature and to the practical application. This study gives the doctor a chance to understand, what dynamics of changes in the expression of differentiation and activation markers of lymphocytes (CD) is the most preferable for the patient. This information will allow him to make a therapeutic decision aimed at increasing the effectiveness of treatment, to share information to improve patient care such as patient electronic health records and facilitate consultations and referrals.

The Research Aim

The aim is to examine the effectiveness of MSC-428 action, used simultaneously with neoadjuvant chemotherapy for breast cancer treatment.

Tasks

1. To determine the initial parameters of the malignant neoplasm of the mammary gland in women using physical, mammography and cytological research methods.
2. To investigate the state of the vital systems of the body before the treatment onset in patients with breast cancer (ECG, complete blood count, proteinogram).
3. To evaluate the results of neoadjuvant chemotherapy in patients with breast cancer (control group) using physical, mammography and histological (degree of pathomorphosis) research methods.
4. To study the results of neoadjuvant chemotherapy in patients with breast cancer during MSC-428 intake by using physical, mammography and histological (degree of pathomorphosis) research methods.
5. To assess the degree of toxicity of neoadjuvant chemotherapy that is conducted with and without MSC-428.
6. To monitor immunological parameters.

The volume of research: 2 groups of patients with breast cancer:

Group 1 (30 people) - conducting neoadjuvant chemotherapy with Mercurid intake (study group).

Group 2 (30 people) - conducting neoadjuvant chemotherapy without Mercurid intake (control group).

Materials and methods: 60 patients with locally advanced breast cancer were examined in the research.

The control group consisted of 30 women who underwent neoadjuvant polychemotherapy (NPChT) according to the AC scheme (Adriablastini+Cyclophosphamide) as the first stage of the treatment.

The study group also included 30 women, but besides neoadjuvant polychemotherapy according to the AC scheme, they took the drug Mercureid, 7 g as a single dose three times daily, in the intervals between I and II, II and III courses of NPChT.

As for the age groups, the patients were divided as follows:

Control group (only chemotherapy - CH): 31-40 years old-9 (30%); 41-50 years old-6 (20%); 51-60 years old-11 (36,7%); older than 60 years old - 4 (13,3%).

Study group (MCS-428 + CH): 31-40 years old-6 (20%); 41-50 years old-8 (26, 7%); 51-60 years old-9 (30%); older than 60 years old - 7 (23,3%) **(Figure 1)**.

In both groups, the following concomitant pathology prevailed:

1. 21 patients (70%) in the control group and 19 patients (63.3%) in the study group had changes in the cardiovascular system;
2. 5 patients (16.7%) in the control group and 6 patients (20%) in the study group had diseases of the gastrointestinal tract;
3. 5 patients (16.7%) and 4 (13.3%) patients respectively had lower-limb varicose veins **(Figure 2)**.

As for TNM stage, the patients were distributed as follows

In Control group (CH): T1N1Mo- 1 (3,3%), T2NoMo- 9 (30%), T2N1Mo- 4 (13,3%), T2N2Mo- 3 (10%), T3NoMo-5 (16,7%), T3N1Mo- 3 (10%), T4N1Mo-1 (3,3%), T4N2Mo-4 (13,3%).

In Study group (MCS-428 + CH): T2NoMo-6 (20%), T2N1Mo-5 (16,7%), T2N2Mo-4 (13,3%), T3NoMo-6 (20%), T3N1Mo-3 (10%), T4N1Mo-3 (10%).

Control group (chemotherapy only) and study group (MCS-428 + chemotherapy) was representative **(Table 1)**.

The second stage of treatment included operation. The volume and type of surgical treatment depended on the degree of tumor metastases reduction in regional lymph nodes.

Results

The results of the treatment were evaluated at the end of the 4th course of NPChT. 11 patients in group I and 8 patients in group II had a complete tumor response, 16 patients in group I and 13 patients in group II had a partial tumor response (more than 50%); 2 patients in group I and 5 patients in group II had a partial

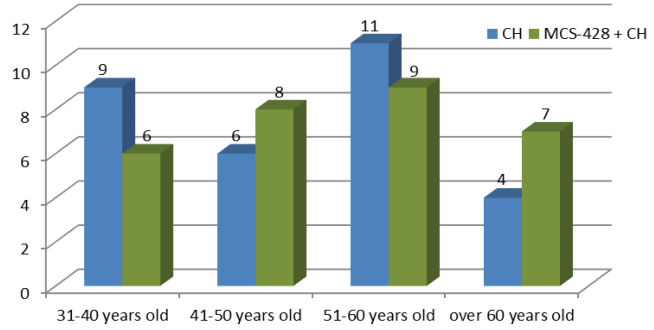

Figure 1 Age Distribution of patients.

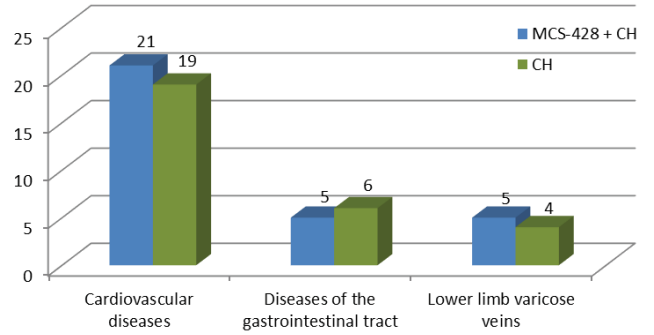

Figure 2 The distribution of patients in the control group for comorbidities.

Table 1 The distribution of patients for TNM stage.

Stage of the Disease (TNM)	Number of Patients	
	MCS-428+CH	CH
T1N1M0	0	1
T2N1M0	6	9
T3N1M0	5	4
T4N1M0	4	3
T5N1M0	6	5
T6N1M0	3	3
T7N1M0	3	1
T8N1M0	3	4

tumor response (less than 50%); the stable disease was observed in 1 patient in group I and in 4 patients in group II.

Group I - MSC-428 + Chemotherapy (CH),

Group II - Chemotherapy (CH).

Histological examination of the surgical material determined the degree of therapeutic pathomorphosis in the tumor. The results of histological examination are presented in **Table 2 and Figure 3**.

7 patients (23.3%) in MSC-428 + Chemotherapy group and 4 patients (13.3%) in the Chemotherapy group had a pathologic complete response of the primary tumor (medical pathomorphosis of grade IV), $p<0.05$ **(Table 3 and Figure 4)**.

The III degree of therapeutic pathomorphosis (necrosis, fibrosis, single degenerative cancer cells) was recorded in 14 patients

Table 2 Tumor response to the treatment.

Effect of Therapy	Number of patients			
	MCS-428+CH		CH	
	Quantity	Share	Quantity	Share
Complete Response, CR	11	36.70%	8	26.70%
Partial Response, >50%	16	53.30%	13	43.30%
Partial Response, <50%	2	6.70%	5	16.70%
Stable Disease, SD	1	3.30%	4	13.30%

Effects of Therapy	Share of patients	
	MCS-428+CH	CH
Complete Response, CR	36.70%	26.70%
Partial Response, >50%	53.30%	43.30%
In general	90.00%	70.00%

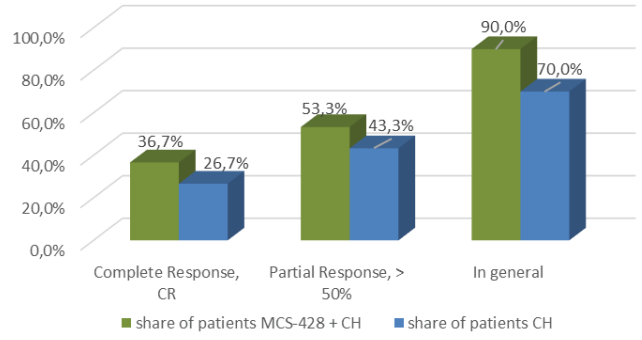

Figure 3 Tumor response to the treatment.

Table 3 The degree of therapeutic pathomorphosis.

Degree of Therapeutic Pathomorphisis	Number of Patients			
	MCS-428+CH		CH	
	Quality	Share	Quality	Share
I	1	3.30%	5	16.70%
II	8	26.70%	12	40.00%
III	14	46.70%	9	30.00%
IV	7	23.30%	4	13.30%

Degree of Therapeutic Pathomorphosis	Share of Patients	
	MCS-428+CH	CH
III	46.70%	30.00%
IV	23.30%	13.30%
In general	70.00%	43.30%

(46.7%) and in 9 patients (30%), respectively, p<0.05. In general, when using pathological evaluation criteria, which include medical pathomorphosis grade III-IV, the effectiveness of the treatment was 70% in the study group and 43.3% in the control group, p<0.05. During chemotherapy the patients of group I (MSC-428 + Chemotherapy), who took MSC-428 medicine, had less obvious adverse reactions compared with group II (Chemotherapy). 56.3% of patients of group I had nausea and vomiting, 14.7% had temperature rising, 5.7% had leukopenia, 61.3% had alopecia II-III stage, p<0.05. In group II, the results were the following: 90.4%, 33.3%, 16.7% and 93.5% respectively, p<0.05. Simultaneously, in patients taking MSC-428, the signs of cancer intoxication were slightly expressed: the appetite was present and the symptoms of depression were less obvious.

Statistical analysis

The univariate significance of differences in marker expression was appraised by Student's t-test for binary or categorical covariates, or by Spearman's rank correlation for ordered covariates. The specific OS and DFS were estimated using the Kaplan-Meier method and comparison between study groups was performed with the log-rank test. The survival time was measured from the date of diagnosis to the date of death or date of last follow-up. In all the tests, the significance level was set at 0.05 and all tests were two-sided. The statistical analyses were performed using the Software Stat Soft Inc. STATISTICA for Windows ver.7.0 A.

Immunological indicators

Dynamics of changes in lymphocyte CD3, CD4, CD8 and CD4 / CD8 index: T-lymphocytes are morphologically indistinguishable from B-lymphocytes. These cells are differentiated by the expression of marker molecules on their surface. The common marker for all varieties of these T-lymphocytes, which is absent in other cells, is TCR-CD3 molecular complex [11]. Detection of CD3-constant molecules which are common to all types of T-lymphocytes-is used to identify T-cells. CD3 was discovered in 1979 with the help of monoclonal antibodies. The selection of T-lymphocytes in CD8+ and CD4+ is extremely important because CD8 + T-cells (T-killers) form a cytolytic molecular complex that ensures the functioning of this T-cell as a cytotoxic T-lymphocyte [12].

In CD4 + T-lymphocytes (T-helpers), intracellular mechanisms that are necessary for performing the "helper" function, first of all-the ability to produce a large amount of cytokines upon activation, are formed. As a result, T cells differentiate into functionally complete subpopulations of cytotoxic and helper T lymphocytes [13].

The concept of cancer immunoediting provides critical information about the two functions of the immune system at the onset and during the development of cancer. However, the

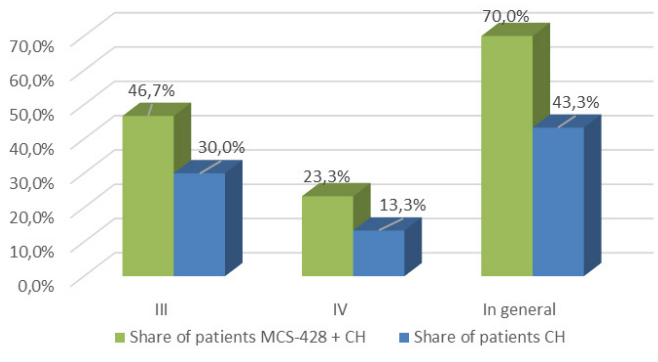

Figure 4 The degree of therapeutic pathomorphosis.

dynamics and role of CD4+ and CD8+ T cells in the pathogenesis of breast cancer remain unclear.

A new consensus has emerged on CD4+ helper T cell and its role in facilitating and mediating sustained anti-tumor responses. The importance of this cell population has emerged, in part due to advances in fundamental immunology and its application in cancer, as well as the massive investment in translational clinical science brought about by industry focus on the PD-1/PD-L1 class of therapeutic antibodies.

To date, most groups working at corralling T cell responses in oncological settings have focused on CD8+ killer T cell, showing at times breathtaking effect, especially in blood cancers like B-cell Lymphoma. This includes approaches utilizing monoclonal antibodies and autologous cells, such as CAR T and TCRs. One challenge with CD8+ T cell is that often, during cancer evolution and progression, one of several mechanisms are coopted to limit the ability of CD8+ T cells to control tumor cells. This includes an induced behavior in tumors, where tumor-specific CD8+ T cells become exhausted and no longer capable of exerting an anti-tumor response. CD4+ helper T cells are critical to providing the signals necessary for sustained CD8+ mediated responses. Furthermore, CD4+ helper T cells are capable of exerting direct anti-tumor activity. To sum up, this is a critical population of cells whose presence not only correlates to improved responses, but also has a direct biochemical link to other important cell populations that further drive cell killing [14].

The study found that the patients had a decrease in the content of lymphocytes with phenotype CD3 + and CD8 +, as a result of tumor intoxication and the effects of chemotherapy. Due to a decrease in the level of CD8 + - lymphocytes, CD4/CD8 index was increased. In the patients with initially reduced number of CD3 + and CD4 + cells, these indicators were fully restored, but MSC-428 intake did not have a stimulating impact on the indicators of the patients with initially normal number of T-lymphocytes and T-helper cells. MSC-428 intake resulted in increasing the number of lymphocytes from 1,3 ± 0,4 to 0.4 to 2,4 ± 0,9, p<0,05.

In the control group (chemotherapy / CH), the changes were not so obvious-1,4 ± 0,3 to 1,5 ± 0,7 respectively, p<0,05. The most significant changes were connected with the increase in the number of CD3 + and CD8 +, as well as the normalization of the immunoregulatory index CD4 / CD8 **(Table 4 and Figure 5)**.

Dynamics of change in the number of lymphocytes with the phenotype CD16 (NK cells): Currently, neoadjuvant chemotherapy is one of the most commonly used treatment strategies for newly diagnosed patients with breast cancer. However, resistance to chemotherapy and recurrence continue to be a clinical problem [15]. The previous studies showed that a decrease of the infiltration of NK cells into tumor tissue may be a prognostic marker for the failure of chemotherapeutic treatment of breast cancer [16]. Natural killers or NK are large granular lymphocytes. They make up 5% of lymphocytes in the peripheral blood. NK cells do not undergo differentiation in the thymus; they go from the bone marrow into the bloodstream and then migrate to tissues where they carry out innate immune defence, called natural cytotoxicity, which is very important for protecting the body from tumors.

Table 4 Dynamics of changes in lymphocyte CD3, CD4, CD8 and CD4/CD8 index.

Index	Norm	MCS-428+CH		CH	
		Before	After	Before	After
Lymphocytes	1.5-3.0X106	1.3 ± 0.4	2.4 ± 0.9	1.4 ± 0.3	1.5 ± 0.7
T-lymph CD3	800-2000	712 ± 112	1234 ± 165	791 ± 106	853 ± 273
T-lymph CD4	400-1200	589 ± 137	591 ± 173	591 ± 173	681 ± 154
T-lymph CD8	100-700	112 ± 23	108 ± 21	108 ± 21	161 ± 54
CD4/CD8	04-Feb	4.5 ± 1.2	4.3 ± 0.7	4.3 ± 0.7	4.1 ± 0.4

The cytoplasm of NK cells has numerous granules containing perforin and granzymes. In addition, NK cells produce TNF-α, which can induce apoptosis of target cells. NK-cell deficiency worsens as the tumor progresses and depends on the clinical stage of the disease [17].

At therapy onset, 68% of patients had an altered number of NK cells (CD16). When MSC-428 was taken, there was a significant increase in the expression of NK cells (CD16) compared with CH group (MSC-428 - 76%, CH group - 34%) **(Tables 5, 6 and Figures 6, 7)**.

Dynamics of changes in the number of lymphocytes with phenotype CD25, CD95: The past years have been characterized by an increasing interest of researchers in the process of programmed cell death, apoptosis [18]. In the immune system, apoptosis is considered as a key mechanism of regulation of T-cell. The high cell readiness for apoptosis is accompanied by the expression of the membrane glycosylated protein APO-1 / Fas (Fas receptor, Fas-R), whose interaction with a specific ligand (Fas-L) initiates the process of apoptotic death. The recent studies have shown that violation of lymphocyte activation, cytokine status (caused not only by a tumor, but also by chemotherapy) is accompanied by a change in the expression of CD95 and is connected with abnormal apoptosis of immunocytes [19]. In this regard, an estimation of the number of CD95+ expressing lymphocytes can be a valuable addition to the characterization of the immune status and has a prognostic value [20].

Spontaneous apoptosis of T-lymphocytes in the bloodstream of patients with serious tumors is a constantly observed phenomenon. Circulating CD8+ T lymphocytes are predominantly targets for apoptosis. Obviously, Fas expression is increased in CD3+ cells which experience chronic antigenic stimulation, as in the case of cancer patients, where Fas expression is found in almost all T lymphocytes.

In case of patients with breast cancer, breast carcinoma may be the source of FasL [21]. There is evidence in the scientific literature that breast tumors overexpress FasL on the cell surface and this overexpressed FasL is responsible for T-cell apoptosis. An additional emphasis is placed on the observation that, even the patients with Stage I disease, when they were diagnosed and no signs of disease were fixed, had a high proportion of T cells undergoing apoptosis.

As the enhanced apoptosis of immune cells leads to a lack of antitumor effector cells, the search of means for protecting T cells from apoptosis in cancer patients is of a great practical

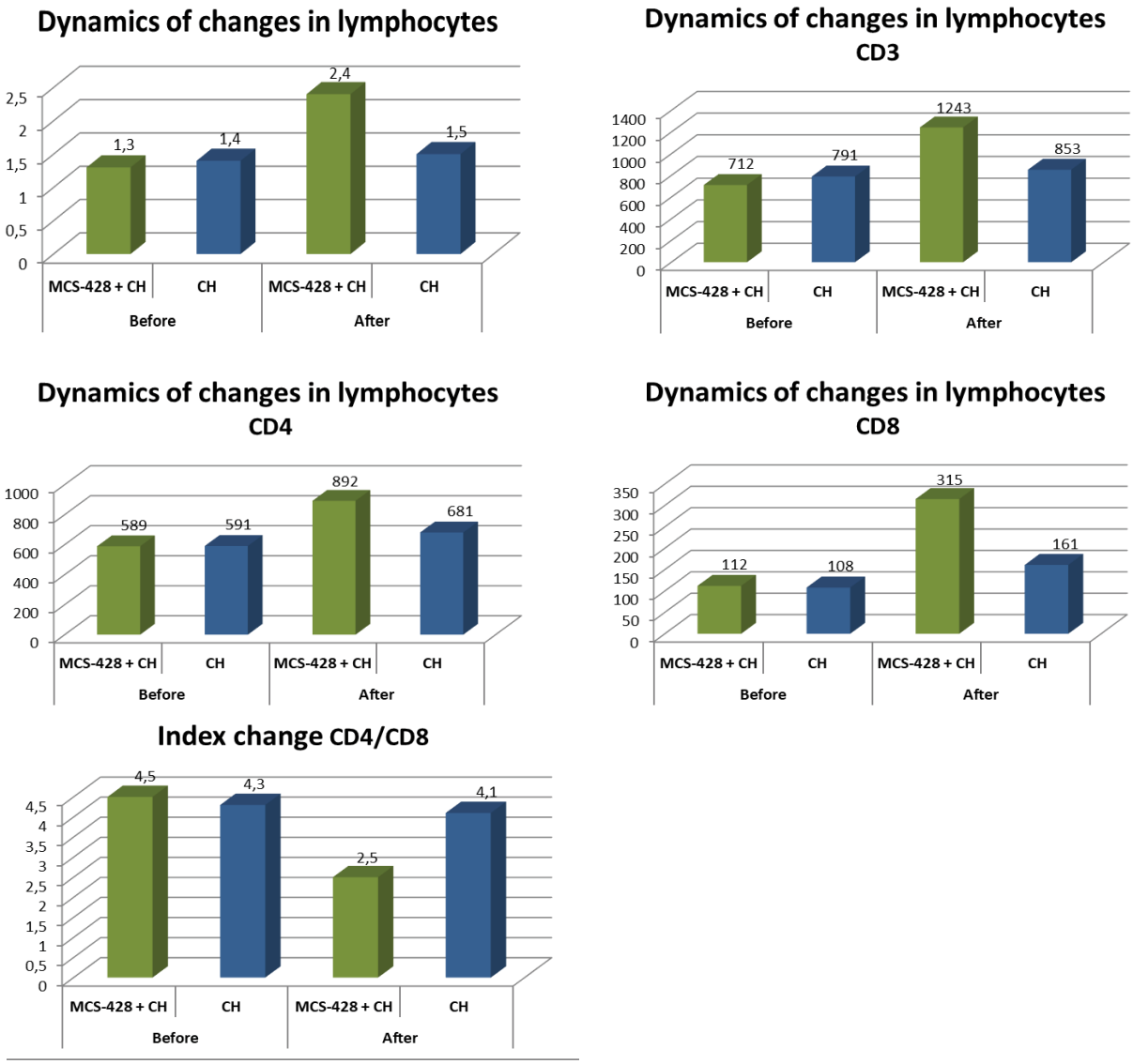

Figure 5 Dynamics of changes in lymphocyte CD3, CD4, CD8 and CD4 / CD8 index.

Table 5 Dynamics of changes in the number of lymphocytes with phenotype CD16.

No	Index Natural killers, CD16	Norm	Before	After
1	MCS-428+CH	10-20%	7.4 ± 2.5	13.2 ± 1.2
2	CH	10-20%	7.3 ± 1.8	9.5 ± 1.8

Table 6 The number of patients who recovered to the normal killer activity of lymphocytes (CD16), in %.

No	Index Natural killers, CD16	Before	After
1	MCS-428+CH	28%	76%
2	CH	27%	34%

interest [22]. The expression of CD25 + on CD3 + T cells appears to be connected with the sensitivity or resistance of the patient's T cells to apoptosis [23]. The obtained data show that the normal

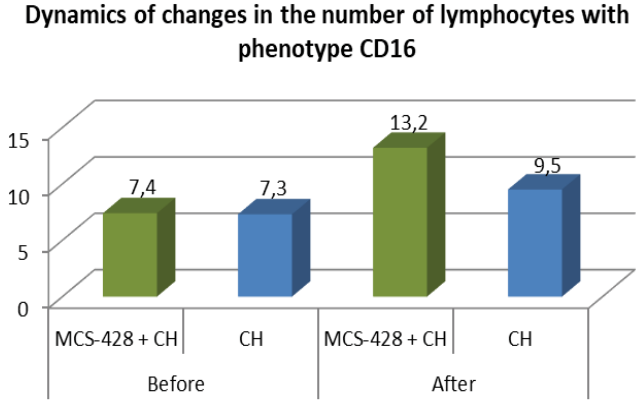

Figure 6 Dynamics of changes in the number of lymphocytes with phenotype CD16.

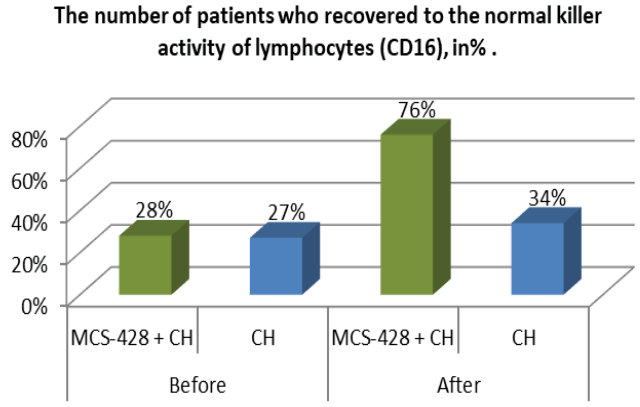

Figure 7 The number of patients who recovered to the normal killer activity of lymphocytes (CD16), in %.

expression of CD25+ on T cells in patients with breast cancer, as a rule, serves to protect these cells from apoptosis. The presence or absence of IL-2Rα on a T-cell is crucial for its response to IL-2, which can act both as a growth factor and a lethal cytokine depending on the cellular microenvironment. Prolactin, which is known to be able to modulate the function of T-lymphocytes and act in conjunction with IL-2, probably also, affects their survival [24].

In the study, the majority of patients (87%) had an increased expression of CD95 + and a decreased expression to IL-2 (CD25) that is characteristic not only of Th2 variant of the immune system response, but also of hyperproduction of pro-inflammatory cytokines [25]. CD95 (Fas or APO1) is a transmembrane glycoprotein belonging to the tumor necrosis factor family and this molecule binding to the Fas ligand leads to the induction of the programmed cell death of CD4+, CD8+, CD16+ cells. The high level of CD95+ and TNFα result in apoptotic death of activated lymphocytes [26]. In the group that took MSC-428, there was an increase in the expression of lymphocytes with phenotype CD25 + and a decrease in CD95 + that correlates with the growth of IL-2 production where IL-2 is a cytokine that enhances the cytolytic function of T-killers and NK cells, increases the production of perforin and interferon-gamma by these cells. The decrease in the expression of marker CD95+ protects the lymphocytes from apoptotic death that resulted in the increase in the number of CD4+, CD8+, CD16+ cells, compared with the control group **(Table 7 and Figure 8)**.

Phagocytosis: At present, phagocytosis is shown to be involved in the elimination of cells that died as a result of apoptosis, damaged by chemotherapy [27]. Tumor cells are also subject to phagocytosis and, to protect against phagocytosis, they don't only deplete the functional property of macrophages, but also overexpress CD-47, which acts as a "don't eat me" signal for the macrophages of the immune system [28]. The way in which breast cancer cells increase the expression of CD47 is in the inflammatory pathway of TNF that is considered to be the preceding stage of NF-κB activation. Our previous studies showed the ability of MSC-428 molecules to reduce TNF overexpression.

This study has shown that patients with breast cancer have a decrease in the absolute number of monocytes and phagocytic activity.

The study has revealed the stimulating effect of MSC-428 on the phagocytic immunity providing an increase in phagocytosis of tumor cells and the body's ability to eliminate cells that have undergone apoptosis as a result of chemotherapy [29]. The determination of neutrophil phagocytic activity using microscopic counting of phagocytic cells and phagocytic objects allowed us to find out a statistically significant increase in phagocytic index in patients who took MSC-428 from 45% to 76%, $p<0.05$. In CH group, the changes in these indicators were insignificant from 42% to 49%, respectively, $p<0.05$ **(Tables 8, 9 and Figures 9, 10)**.

The phagocytic activity reveals the dynamics of the host tumor interface. This method is used alone or combined with other methods as an indicator of the extent or activity of the disease. We conclude that monocyte phagocytic function can be used as an additional prognostic factor in breast cancer monitoring.

Dynamics of changes in the number of lymphocytes with the phenotype CD38: CD38 encodes a membrane protein that participates in cell adhesion and catalyzes the formation of cyclic ADP-ribose. The previous studies have revealved that the cells in which the stable expression of CD38 occurs are present in the tumor foci of patients. The previous studies have shown that the level of CD38 increases with lung cancer, stomach cancer and breast cancer [30].

CD38 increases in breast carcinoma, starting with the second stage of tumor growth, and remains high in the subsequent stages, regardless of the metastasis location. The highest content of the antigen under study is observed with a combined histological form of the tumor. The statistically proven high levels of CD38 are kept both in single and multiple lesions [31]. A high concentration of CD38 antigen is characteristic of any tumor diameter.

There is a model for the participation of CD38 antigen in limiting the migration of mononuclear cells from the vascular bed to the tissue space, and, accordingly, to the site of tumor localization [32]. The presented model reflects one of the ways by means of which the mechanisms of the tumor escape from immune surveillance are formed [33].

Thus, a high level of CD38 antigen can be considered as one of the factors of tumor escape from the anti-tumor response on the part of the immune system [34]. CD38 correlates with violation of the signaling pathway of phosphatidylinositol 3-kinase (PI3K) / Akt. Normalization of CD38 occurs when being remission, thus, it is advisable to use it as a monitoring prognostic indicator in the treatment of breast carcinoma [35] **(Table 10)**. MSC-428

Table 7 Dynamics of changes in lymphocytes CD25, CD95.

Index	Norm	MCS-428+CH		CH	
		Before	After	Before	After
CD25	10-18%	9.3 ± 1.5	14.2 ± 1.8	9.7 ± 1.4	10.9 ± 1.3
CD95	10-20%	25.6 ± 3.2	17 ± 1.9	24.8 ± 3.6	22.4 ± 3.1
CD25/CD95	0.5-1.8%	0.3 ± 0.07	0.83 ± 0.08	0.39 ± 0.06	0.48 ± 0.05

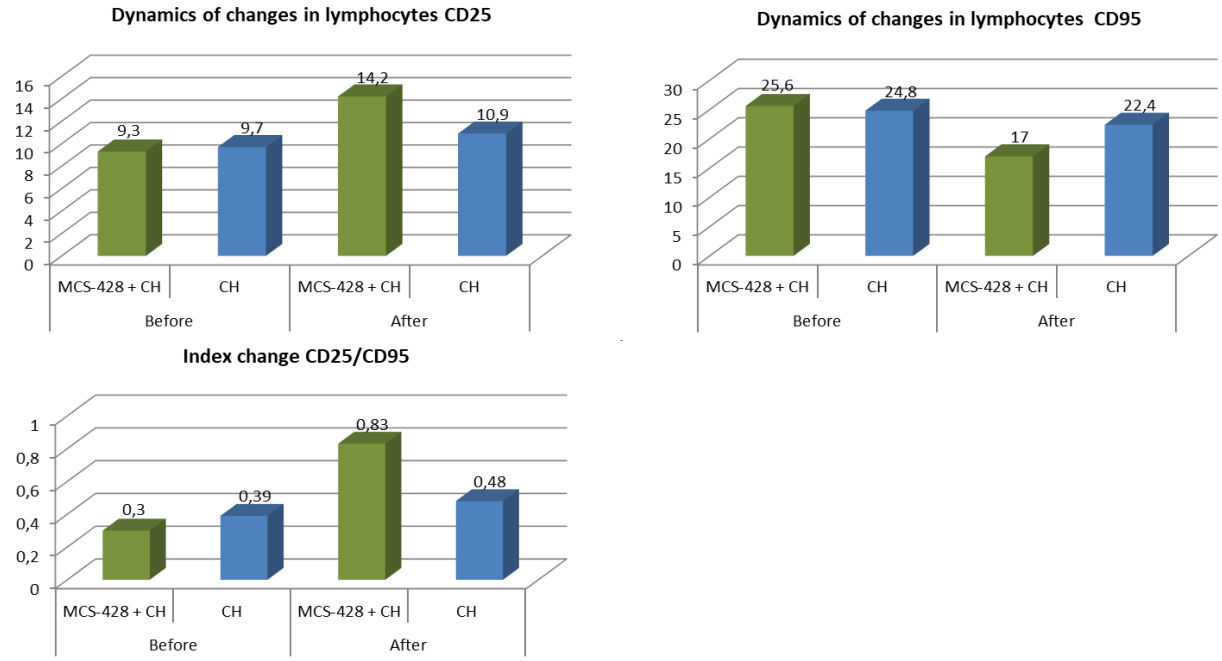

Figure 8 Dynamics of changes in lymphocytes CD25, CD95.

Table 8 Dynamics of changes in phagocytosis.

No	Index Phagocytosis	Norm	Before	After
1	MCS-428+CH	1600-4000	1618 ± 312	2870 ± 196
2	CH	1600-4000	1657 ± 298	1945 ± 187

Table 9 The number of patients in whom phagocytic activity returned to normal, in%.

No	Index Phagocytosis	Before	After
1	MCS -428+CH	45%	76%
2	CH	42%	49%

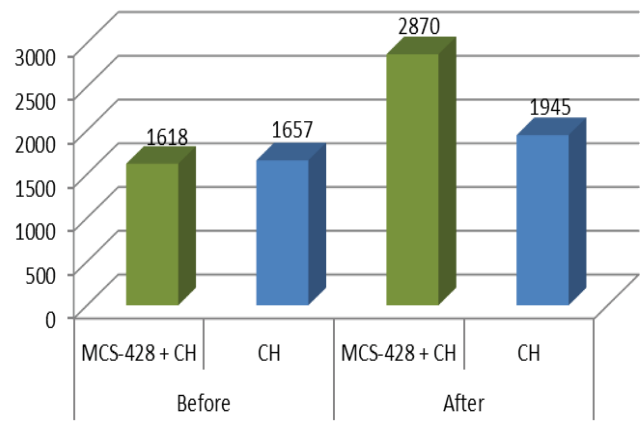

Figure 9 Dynamics of changes in phagocytosis.

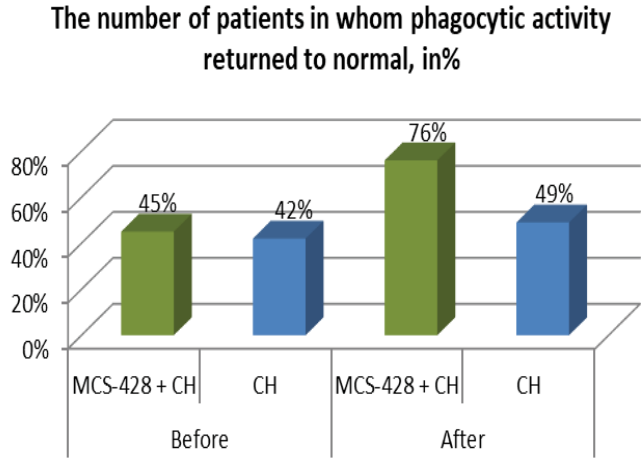

Figure 10 The number of patients in whom phagocytic activity returned to normal, in%.

molecules are CD38 antagonists, as a result, CD38 expression is decreased that is considered to be an indicator of the therapy effectiveness **(Figure 11)**.

In the study group, during MSC-428 intake, there was a more obvious decrease of this indicator from 788 ± 213 to 312 ± 116. In the control group, from 801 ± 198 to 592 ± 187, p<0,05. The researchers from the University of Texas MD Anderson Cancer Center (MD Anderson) identified CD38 as a new immune checkpoint protein [35] that works by inhibiting the cytotoxic function of CD8 T-lymphocytes and thereby contributing to resistance to PD-1 / PD-L1 inhibition in cancer. Modern MAbs, such as Darzalex (daratumumab) produced by Janssen pharmaceutical company (Germany), cost about $ 127,000 per a

course of medication. That's why they cannot be widely applied because of the low purchasing power of patients.

The dynamics of changes in the number of lymphocytes with phenotype CD45: CD45, LCA is a common leukocyte antigen which belongs to member of the protein tyrosine phosphatase (PTP) family. CD45 performs an important function in signal transduction into the cell from the T-cell receptor and is presented on the surface of T-cells by various isoforms. One of the functions of CD45 is the binding of T-cell receptor to CD4 or CD8 co-receptors that ensures the efficient signal pass from the antigen into the cell [36]. The decrease in expression of CD45 increases the threshold of sensitivity for TCR/CD3 complex that leads to defects in positive and negative lymphocyte selection, and also potentiates Fas-dependent apoptosis [37].

Studying the relationship of the immune response with the degree of breast cancer prevalence revealed a number of very important regularities: with a decrease in the overall level of leukocyte infiltration (CD45+), the frequency of metastatic lesions of the regional lymph nodes increased significantly [38]. The detection of distant metastases was also significantly negatively correlated with the overall level of the immune response (CD45 +). The overall level of the immune response, assessed by CD45, may serve as an independent factor of favorable prognosis in patients with breast cancer.

The definition of CD45 showed that with a mild reaction, the overall 5-year survival rate was 61.4 ± 9.3%; with moderate - 72.7 ± 7.0% and with severe - 77.9 ± 5.3% (V.P. Letyagin, N.N. Tupitsyn, E.V. Artamonova, RONS named after N.N. Blokhin, Russian Academy of Medical Sciences, Moscow, based on the materials of the VII Russian Oncological Conference).

The contemporary researches specify the central role of CD45 in inducing the expression of a transcriptional inhibitor Bcl-6, which regulates the expression of CTLA-4 [39]. Bcl-6 is a key regulator in Th-cell differentiation and in the regulation of CD4+ and CD8+ T-cell memory [40]. Although it has been demonstrated before that Bcl-6 is transiently expressed in activated T cells and continuously up-regulated in CD8+ effector T cells [41].

CTLA4 or CTLA-4 (cytotoxic T-lymphocyte-associated protein 4), also known as CD152 (cluster of differentiation 152), is a protein receptor that, functioning as an immune checkpoint, downregulates immune responses. CTLA4 is constitutively expressed in regulatory T cells but only upregulated in conventional T cells after activation-a phenomenon which is particularly notable in cancers. It acts as an "off" switch when bound to CD80 or CD86 on the surface of antigen-presenting cells.

Using antagonistic antibodies against CTLA such as ipilimumab (FDA approved for melanoma in 2011) as a means of inhibiting immune system tolerance to tumours and thereby providing a potentially useful immunotherapy strategy for patients with cancer [42]. The 2018 Nobel Prize in Physiology or Medicine was awarded to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation" **(Table 11)**. In MSC-428 + CH group, there was a more intense increase in CD45 marker compared with CH group from

Table 10 Dynamics of changes in lymphocytes CD38.

No	Index Phagocytosis	Norm	Before	After
1	MCS-428+CH	150-600	788 ± 213	312 ± 116
2	CH	150-600	801 ± 198	592 ± 187

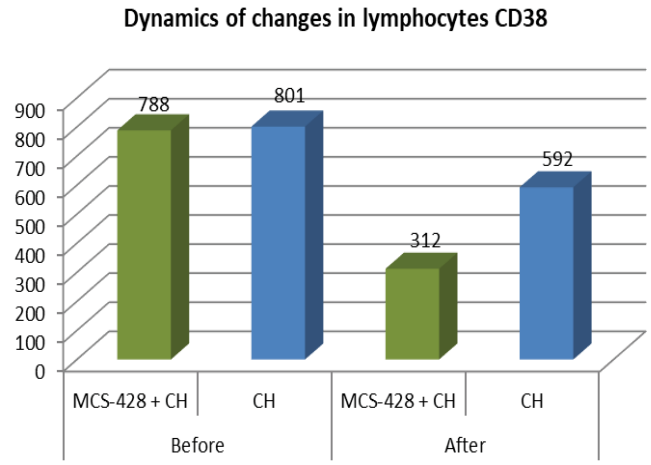

Figure 11 Dynamics of changes in lymphocytes CD38.

11,9 ± 2,1 to 21,4 ± 3,6 and, accordingly, in CH group from 12,3 ± 2,8 to 15,6 ± 2,9, p<0,05 **(Figure 12)**.

Conclusion

1. At the beginning of the study, most patients had lymphopenia. In the group that took MSC-428 + CH, there was an increase in the number of lymphocytes from 1,3 ± 0,4 to 2,4 ± 0,9 (84.6% increase). In CH group, the changes were not so obvious-1,4 ± 0,3 to 1,5 ± 0,7 respectively.
2. CD4 + T-lymphocytes are required for antigen presenting function and active cytokine production. In MSC-428 + CH group, this indicator was 589 ± 137 before the treatment and became 892 ± 198 after the treatment (51.4% increase). In CH group, 591 ± 173 - before the treatment and 681 ± 154 - after the treatment (15.3% increase).
3. CD8 + T-cells (T-killers) form a cytolytic molecular complex that ensures the functioning of T-cell as a cytotoxic T-lymphocyte. In MSC-428 + CH group, this indicator was 112 ± 23 before the treatment and 315 ± 35 after the treatment (181% increase). In CH group, 108 ± 21 - before the treatment and 161 ± 54 - after the treatment (49% increase).
4. According to the changes in the numbers of CD4 + and CD8 + cells the ratio of these cells - the immunoregulatory index CD4/CD8 - also changed. Initially, it was high due to the cell imbalance. After the patients' treatment in MSC-428 + CH group, it became normal: from 4,5 ± 1,2 to 2,5 ± 0,3. In CH group, it remained a little higher due to the imbalance of CD4 + and CD8 + cells and had the indicator 4,3 ± 0,7 before the treatment and 4,1 ± 0,4 after the treatment. The norm is 2-4 %. These changes are statistically significant, p<0.05.

Table 11 Dynamics of changes in CD45 lymphocytes.

No	Index Phagocytosis	Norm	Before	After
1	MCS-428+CH	12-24	11.9 ± 2.1	21.4 ± 3.6
2	CH	12-24	12.3 ± 2.8	15.6 ± 2.9

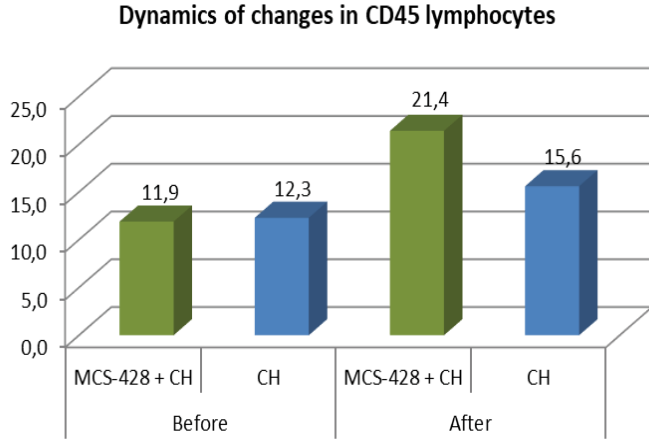

Figure 12 Dynamics of changes in CD45 lymphocytes.

5. The number and activity of NK cells were initially reduced and in MSC-428 + CH group they were 7,4 ± 2,5 before the treatment and 13,2 ± 1,2 after the treatment (78.4% increase). In CH group, they were 7,3 ± 1,8 before the treatment and 9,5 ± 1,8 after the treatment (30.1% increase). The norm is 10 - 20%.
6. Before the beginning of the treatment, the changes in the number of NK cells were observed in 28% of patients of MSC-428 + CH group, after the treatment the recovery to the norm was fixed in 76% of patients of the same group. In CH group, the changes were observed in 27% of patients, the recovery to the norm - in 34% of patients.
7. CD25 as an early activation marker, receptor for IL-2 is expressed in the developing and activated lymphocytes. In MSC-428 + CH group, there was a change from 9,3 ± 1,5 to 14,2 ± 1,8 (52.7% increase). In CH group, respectively, from 9,7 ± 1,4 to 10,9 ± 1,3 (12.4% increase). The norm is 10 - 18%.
8. CD95 expressing lymphocytes were high in both groups. But more intensive recovery occurred in MSC-428 + CH group from 25,6 ± 3,2 to 17 ± 1,9 (71.6% decrease). In CH group, from 24,8 ± 3,6 to 22,4 ± 3,1 (10.8% decrease). The norm is 10 - 20%.
9. The phagocyte system is important from the standpoint of the elimination of the cells died after chemotherapy. But pCHT (preoperative chemotherapy) intake has a suppressive effect on the monocyte and phagocyte link of the immune system. In MSC-428 + CH group, there was a statistically significant increase in phagocytic activity (from 45% to 76%, $p<0.05$). In CH group, the changes in these indicators were insignificant from 42% to 49%, respectively, $p<0.05$.
10. CD38 goes up in breast carcinoma, starting with the second stage of tumor growth and remains high in the subsequent stages. Some researchers offer to use it as a prognostic factor for the therapy success. In MSC-428 + CH group, this indicator decreased from 788 ± 213 to 312 ± 116 (61.4% decrease). In CH group, from 801 ± 198 to 592 ± 187 (26.3% decrease). The norm is 150 - 600.
11. The high expression of CD45 marker correlates with 5-year survival rate of the patients. In MSC-428 + CH group, the dynamics of the increase in CD45 was from 11,9 ± 2,1 to 21,4 ± 3,6 (79.8% increase). In CH group, from 12,3 ± 2,8 to 15,6 ± 2,9 (26.8% increase). The norm is 12 - 24%. The prescription of MSC-428 provides a higher survival rate.
12. In general, when using pathological evaluation criteria, including medical pathomorphosis grade III-IV, the effectiveness of the treatment was 70 % in MSC-428 + CH group and 43.3% in CH group.
13. 7 patients (23.3%) in MSC-428 + CH group and 4 patients (13.3%) in CH group achieved the pathologic complete response of the primary tumor (therapeutic pathomorphism of grade IV).
14. With 51,5 months median follow-up, patients in the study have been followed up for sufficient time, to enable a comparison of long-term post treatment effects of MSC-428 + Chemotherapy relative to only Chemotherapy **(Figure 13)**.
15. During the observation period in the group MSC-428 + Chemotherapy, 4 patients had a recurrence: 2 with distant metastases and 2 with local recurrence.

In Chemotherapy (CH) group, 7 patients had a recurrence: 4 with distant metastases and 3 with local recurrence.

The combination immunotherapy / chemotherapy demonstrates a promising potential for effective cancer treatment by engaging the immune system in the process of tumor destruction. MSC-428 nanomolecules had a multitarget impact on a large group of lymphocyte receptor proteins, demonstrating the properties of mixed agonist-antagonist. Agonists: CD3, CD4, CD8, CD16, CD25, CD45 Antagonists: CD38, CD95.

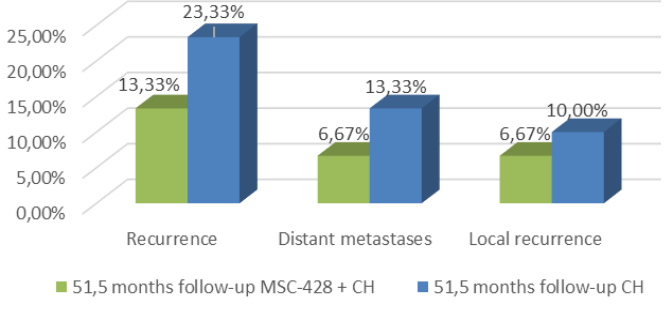

Figure 13 Comparison of long- term post treated effects of MSC-428 +chemotherapy relative to only chemotherapy.

As a result, we have a SMART effect from immunotherapy and as a result, a more effective antitumor response for the patient.

MSC-428 application in the complex therapy:

1. Improves the efficacy and tolerance to chemotherapy;
2. Reduces side effects caused by chemotherapy;
3. Prevents the apoptotic death of immunocytes induced both by tumor and chemotherapy;
4. Increases the number of lymphocytes with phenotype CD3, CD4, CD8;
5. Increases NK cell killer activity, CD8;
6. Restores phagocytosis system compromised by chemotherapy;
7. Reduces the expression of CD38, which is defined as a new immune checkpoint protein that inhibits the cytotoxic T cell activity;
8. Activates CD45, thereby regulating the expression of CTLA-4, which functions as an immune checkpoint, suppressing immune responses.

Final Comments

CD38 and CD45 (CTLA-4) are immune checkpoints, and binding of these inhibitory receptors to their ligands prevents T-cell receptor signaling, inhibits the cytotoxic T-cell function and promotes T-cell apoptosis.

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