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### Results of Multitarget Therapy Anti-PD-1/PD-L1/CD19/CD25/CD38 with Application of MSC-428 Molecules in Patients with Different Oncopathology

# Lukyanchuk Oleg Valerievich<sup>1</sup>, Artemov Alexander Valentinovich<sup>2</sup>, Buryachkovsky Eduard Stanislavovich<sup>3</sup>, Velichko LN<sup>4</sup> and Gusev SN<sup>5</sup>\*

<sup>1</sup>Chief Medical Officer of Odessa Regional Oncology Dispensary, Head of the Department of Reconstruction and Rehabilitation Medicine, Candidate of Medical Sciences, Associate Professor, Ukraine

<sup>2</sup>Candidate of Medical Sciences, Head of the Laboratory of Pathological Anatomy and Tissue Conservation, "The Filatov Institute of Eye Diseases and Tissue Therapy of the National Academy of Medical Sciences of Ukraine", Odessa, Ukraine

<sup>3</sup>Candidate of Medical Sciences, Associate Professor of the Department of Pathological Anatomy, Dean of the International Faculty, Odessa National Medical University, Odessa, Ukraine

<sup>4</sup>Head of Immunological Laboratory, Filatov Institute of the National Academy of Medical Sciences, Ukraine

<sup>5</sup>Author, Developer of New Molecules, Owner of Intellectual Ownership of Molecules, Ukraine

\*Corresponding Author: Gusev SN, Author, Developer of New Molecules, Owner of Intellectual Ownership of Molecules, Ukraine

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### Abstract

The Nobel Prize in Physiology or Medicine 2018 was awarded jointly to James P. Allison and Tasuku Honjo "for their discovery of cancer therapy by inhibition of negative immune regulation".

For more than 100 years scientists attempted to engage the immune system in the fight against cancer. Until the seminal discoveries by the two laureates, progress into clinical development was modest. Checkpoint therapy has now revolutionized cancer treatment.

This mechanism is associated with the appearance on the surface of tumor cells of specific proteins - PD-1/PD-L-1, which prevent contact with the killer lymphocyte receptor.

New molecules MSC-428 (tradename Mercureid) target PD-1/PD-L-1, i.e. affect the immune checkpoints directly. MSC-428 molecules attack 4 target proteins: PD-1/PD-L1, CD19, CD25, CD38, which leads to the normalization of surface antigens - CD3, CD4, CD8, CD16, CD45, CD95 lymphocytes, phagocytosis systems and immunoglobulins of class A, M, G.

The proposed strategy of treatment against PD-1/PD-L-1 with the use of nanocomplex MSC-428 expands the possibilities of influencing immune checkpoints. Through conformational change in the 3-dimensional structure of the receptor proteins PD-1, the drug prevents them from binding to PD-L1 in tumor cells.

This increases the expression of CD25 on T-lymphocytes, normalizes the production of IL-2, which increases the activity of T-killers against tumor cells. The drug reduces the activity of CD19 molecules, which shield tumor antigens and prevent the attack of cytotoxic T cells.

Because of inhibition of ZAP-70 kinase, CD38 expression is reduced, which increases the body's antitumor response and mobilizes the cytotoxic potential of CD8 and CD16 T lymphocytes, preventing the development of tumor cell resistance.

The restoration of their activity largely predetermines the normalization of other lymphocyte subpopulations, in particular, expressing CD3, CD4, CD8, CD16, CD95, as well as the restoration of the phagocytosis system, production of immunoglobulins of class A, M, G. Such a complex effect ensures the construction of a coherent and effective strategy for immunotherapy of patients with oncopathology.

New molecules MSC-428 (tradename Mercureid) have the unique pharmacochemical properties provide an opportunity for multi-purpose therapy directed against several target proteins: PD-1/PD-L1, CD19, CD25, CD38.

Thus, we overcome the shortcoming of traditional therapy with monoclonal antibodies. Because the effectiveness of mAb depends on the degree of affinity of the drug-antibodies to one, highly specific protein receptor (PD-1, PD-L1 or CTLA-4 etc.).

Keywords: PD-L1; CD19; CD25; CD38; MSC-428; Oncopathology

#### Introduction

The study of the immune system functioning when malignant neoplasms appear and start developing is of great practical interest nowadays because despite the advances in the study of the molecular and genetic aspects of oncogenesis, there is still no clear understanding of the role of antitumor immunity, the relations between the man's immune system and developing tumors [1]. There is an opinion that the tumor induces immunosuppression, which can occur in the range: from a weak immune response to full anergy. However, the regularities of the immunological failure formation, the causes and mechanisms resulting in a decrease in the immune system reactivity, have not been clearly defined yet.

Immune detection of a tumor has its own specifics, because tumor cells originate from "the self" and tumor antigens do not quite correspond to the traditional role of "incoming from outside" (if to compare with viruses and bacteria that enter the body from outside). In this connection, it is acknowledged that antitumor immunity constitutes a special paradigm of immune detection of "the self" and "the transformed self". Due to the low immunogenicity of the antigens, a weak and short-term immune response, that does not lead to tumor destruction in vivo, develops. The above-mentioned information makes it possible to consider the study of the immune system functions in the process of tumor growth a very topical issue providing a vital contribution to the solution of immunotherapy problems.

#### How does the tumor avoid immunity attacks?

Tumor cells are able to avoid control and destruction by the immune system, using a series of complex and often overlapping mechanisms that lead to a destruction of key components involved in an effective antitumor response. Cancer cells use immune checkpoints to escape attacks from the immune system to survive [2,3]. In other words, on the surface of the transformed cells special proteins (PD-L1) appear. Contacting with the killer cell receptor (lymphocyte) they inform that they are "not cancerous". The lymphocyte after receiving such a signal does not attack the tumor, it falls into a state of anergy. In this case, cancer cells can freely reproduce in the body.

#### PD-1/PD-L1 Pathway

The PD-1 receptor (Programmed cell death 1; CD279) is expressed on the surface of activated T cells. Its ligands, PD-L1 (B7-H1, CD274) and PD-L2 (B7-DC, CD273) are usually expressed on the surface of dendritic cells or macrophages. PD1 and PD-L1/PD-L2 belong to the family of immune checkpoint proteins that act as co-inhibitory factors which can stop the development of a T-cell response [4-6].

PD1/PD-L1 ensures that the immune system starts activating only at the definite time to minimize the development of chronic autoimmune inflammation. Thus, PD-L1/PD-L2 are located not only on tumor cells, but also on dendritic cells and macrophages - performing an important function of immunological tolerance.

Doctors and patients have already experienced a situation when, on the background of anti-PD-L1/PD-L2 therapy, patients have got type I diabetes (Yale University, immunologist Kevan Herold) [7] or autoimmune attacks on the heart resulted in patients' death (autoimmune attacks on the heart, called lymphocytic myocarditis, Andrew Lichtman, a pathologist at Brigham and Women's Hospital in Boston) [8-12].

When PD-L1 binds to PD-1, an inhibitory signal which suppresses the T-cells proliferation is transmitted to this T-cell. Tumor cells use this pathway as a mechanism for inhibiting the immune response.

#### Drugs that target PD-1 or PD-L1

Over the past 2 years, the anti-PD-1 inhibitors, nivolumab (OPDIVO, Bristol-Myers Squibb) [13] and pembrolizumab (KEY-TRUDA, Merck Sharp and Dohme Corporation) and the anti-PD-L1 atezolizumab (TECENTRIQ, Genentech Oncology) have been approved by the US Food and Drug Administration (FDA) for the treatment of patients with advanced NSCLC who have progressed following first-line therapy or after it. The European Medicines Agency (EMA) has approved Nivolumab and Pembrolizumab judg-ing by the same characteristics.



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#### Price of PD-1/PD-L1 inhibitors

Drug	Target	Side effects	Price, 1 injection	Price, 1 year
Pembolizumab (Keitruda)	PD-1	Hepato, nephro, cardiotoxicity	\$7 125	\$242 000
Atezolizumab (Tecentriq)	PD-L1	Hepato, nephro, cardiotoxicity	\$7 140	\$257 000
Bavencio (Avelumab)	PD-L1	Hepato, nephro, cardiotoxicity	\$13 000	\$156 000

#### **Overview of anti-PD-1/PD-1 therapy**

Drugs based on monoclonal antibodies (English, MAbs) form TOP-6 sales of world pharma. But in spite of their commercial attractiveness, they show limited efficiencies [14] from 10 up to 20% that is considered quite a small indicator of efficiency taking into account their cost.

Despite the clinical success of immunotherapy against PD-1/ PD-L1, some patients do not respond to these therapies. The reasons for immunity insensitivity are connected with several factors, such as insufficient infiltration of activated CD8+ T-cells in the tumor microenvironment, tumor heterogeneity, hypoxia and variability of mutations of specific oncogene pathways.

### The reasons for low effectiveness of MAbs following anti-PD-1/PD-L1 therapy

- 1. Monoclonal antibodies (MAbs) are directed against only one antigen (PD1 or PD-L1, etc).
- 2. These drugs cause dangerous side effects skin, gastrointestinal, hepatic, endocrine, renal, cardiotoxic [15].
- 3. They have a clear immunogenicity. The immune system produces blocking antibodies against them that reduces the therapy effect and activates B-lymphocytes. First of all, these are CD19 antibodies, which block tumor antigens [16].
- MAbs inhibit the activity of an important CD25 protein [17], which ensures the proliferation and differentiation of T-lymphocytes into CD8 or CD16 cytotoxic cells.
- 5. The researchers from the University of Texas MD Anderson Cancer Center (MD Anderson) identified CD38 as a new immune checkpoint protein [18] 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.

### Innovative molecules to increase the effectiveness of anti-PD-1/PD-1 therapy

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The proposed strategy to improve therapy against PD-1/PD-L-1 with the help of "MSC-428" molecules enhances the possibilities of immune checkpoint therapy significantly:

- 1. "MSC-428" is capable of causing conformational changes in the 3-dimensional conformation of the receptor proteins PD-1, that prevents their binding to the PD-L1 tumor.
- 2. "MSC-428" increases the active expression of CD25 T-cells, normalizes the production of IL-2 [19], that increases the ability of T-killers to kill tumor cells and stimulates the production of performs and granzymes.
- "MSC-428" reduces the increased activity of CD19, which screen tumor antigens and prevent the attack of cytotoxic T-cells [20].
- "MSC-428" molecule inhibits ZAP-70 kinase, reduces CD38 overexpression [21], that increases the antitumor body reactions and mobilizes the cytotoxic potential of CD8, CD16 T-lymphocytes, and prevents the development of resistance to tumor cells [22,23].

## Competitive advantages of MSC-428

### Targeting Anti-PD-1/PD-L1/CD19/CD25/CD38 THERAPY

- 1. Nanomolecules MSC-428 (1.2 nm size) are considered to be antagonists of 4 target proteins: PD-1/PD-L1, CD19, CD25, CD38.
- 2. Tactics of multitarget therapy directed at 4 target proteins contributes to a better therapeutic result.
- 3. It allows overcoming numerous cases of MAbs resistance with therapy only against target protein 1 (PD-1/PD-L1).
- This medicine, in accordance with the classification of substances for toxicity, belongs to class IV, low-toxic substances, 501 < LD50 < 5000 mg/kg.</li>

<b>Molecul</b> e	Target	Side effects
MSC-428	PD-1/PD-L1/	Safe, lowest IV toxicity class
	CD19/CD25/CD38	
	therapy	

#### **New molecules**

Various types of nanoparticles have been used to deliver cancer drugs. The company "Mercureid pharmaceutical" developed new nanosized molecules which have the name "MSC-428". As for their shape, the molecules represent a tetrahedron. They have an ultra-small size of 1.2 nm. For example, the size of hepatitis C virus is 60 nm, the size of leukocyte is 13,000 nm. The use of a metal nanoparticle with amino acids as "anchor molecules" provides a strong adhesion to cell receptors. This thing allows implementing targeted therapy.

#### **Know How in Immune Checkpoint Therapy**

Although the scientific studies specify the need for the therapy of immune checkpoints, but in practice it is restrained by the high

cost of brand drugs, the risk of side effects and limited effectiveness caused by the drugs influence only on one target protein. The Ukrainian innovative molecule MSC-428 (certificate of registration for a medicine №UA6098/01/01) which is a modulator of activity of 4 target proteins PD-1/PD-L1, CD19, CD25, CD38 can become an alternative to the costly drugs. The active substance MSC-428 is a low molecular coordinating compound of chelating type in which the central metal atom is bound to L-cysteine molecules. Having a molecular weight of 440 Da, the molecule size is 1.2 nm. The supersmall size allows to be integrated into the active cell receptor binding site, and the presence of a metal atom predetermines conformational changes in the spatial structure of the receptor protein. As a result, complementarity (a correspondence between ligands and receptors belonging to the immunoglobulin superfamily) is lost. Thus, despite the differences in the amino acid sequence of the PD-1/PD-L1, CD19, CD25, and CD38 receptors, they are united by the presence of a three-dimensional structure necessary for the specific recognition of their ligand. The uniqueness of this structure is determined by the disulfide (-S-S-) bonds of cysteine molecules.

Molecules MSC-428 break disulfide bonds (-S-S-) in the receptor, and the metal ion causes a conformational rearrangement of a three-dimensional structure of the receptor [24,25], thereby violating complementarity, i.e. spatial correspondence of the receptor to its ligand. As a result, the receptor is unable to attach the ligand, and the destructive signals do not come into the cell. In such a way, MSC-428 molecules perform multitarget therapy directed against the four target proteins PD-1/PD-L1, CD19, CD25, CD38 that predetermines a greater efficacy comparing with the conventional therapy of immune checkpoints where only one target protein is inhibited.

#### The Purpose and Objectives of the Research

The main purpose of the research was to define the regularities of the functional and phenotypic shift in the subpopulations of blood lymphocytes in patients with various oncological diseases. In this regard, the following tasks were put forward:

- 1. To characterize the features of the functional state of blood T and B lymphocytes in patients with lung cancer, stomach cancer, breast cancer, colorectal cancer conducting the comparative study.
- 2. To study the phenotypes of blood lymphocytes as for the expression of differentiating CD3, CD4, CD8, CD16, CD19 and activating markers CD25, CD38, CD45, CD54, CD95 in patients with lung cancer, stomach cancer, breast cancer, colorectal cancer.
- 3. To compare the dynamics of changes in the level of proliferative activity of lymphocytes and expression of cell surface antigens at different stages of the disease examining the patients with various oncological pathologies. To assess the functional shifts of the main subpopulations of T and B lymphocytes.

- 4. To characterize the changes in the phenotype of patients' blood lymphocytes depending on the stage of the disease and localization of the tumor. To assess phenotypic changes in lymphocytes of oncological patients depending on the course and outcome of the disease.
- 5. To compare the functional and phenotypic changes in blood lymphocytes for various oncological diseases and to determine the most characteristic systemic shifts in the functions of the immune system that occur during the tumor development.
- 6. To reveal the specifics of the state of the immune system in oncological patients in comparison with the diseases of a different nature.

The trial included patients who gave written Informed Consent to participate in the study and who met the inclusion/exclusion criteria.

Clinical laboratory and instrumental studies of patients were carried out in accordance with the survey scheme.

#### The scope of the research

Three groups of patients took part in the research work.

This study was conducted in accordance with the Law of Ukraine "On medicines" and ethical principles of the Helsinki Declaration. Patients who are potential participants in the study were informed of the nature of the test, the study drug, as well as the possible risk associated with taking the drug. Each patient was provided with written information about the study drug and the study conducted, contained in the "Information for the patient". During the study, patients of all groups were recommended a fruit-and-milk-vegetable diet. At the time of the study, drinking alcohol and coffee were excluded. During the treatment, the following preparations were not allowed: biologically active substances, immunostimulants.

**Mercureid group:** Mercureid group consisted of 192 patients, who had been previously treated with different combinations of antitumor drugs. These patients received Mercureid as the only possible therapy after all the other attempts of using standard chemotherapy were found invalid. The patients' age period was in the range from 28 to 74 years old, the average age was 56.5 years old. There were 72 men and 120 women. 57 patients had a radiation therapy in the past. The patients' general condition according to the criteria of the World Health Organization (WHO) was estimated as 2.9 on average. The duration of the drug use was 2 months. The analyzes were done on the first day of treatment and on the 60th day of treatment.

**Mercureid+ ChT group:** Mercureid+ ChT group consisted of 174 patients. The patients' age period was in the range from 29 to 75 years old. The average age was 50.1 years old. There were 68 men and 106 women. All patients suffered a common stage of oncologi-

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cal disease and previously they had received from 2 to 19 courses of chemotherapy; 52 patients had received radiation therapy. The patients' general condition according to the criteria of the World Health Organization was estimated as 2.6 on average. The patients were taking Mercureid for 2 months, in the breaks between the courses of chemotherapy and radiation therapy. Blood sampling was carried out at the beginning and at the end of Mercureid intake.

**The Control group:** Control group consisted of 164 patients. The patients' age period was in the range from 32 to 81 years old. The average age was 54 years old. All patients received from 4 to 21 courses of chemotherapy according to their diagnosis and therapy protocol. 62 patients had radiation therapy. These patients did not receive immunotropic therapy. The patients' general condition according to the criteria of the World Health Organization was estimated as 2.8 on average. To assess the ability to restore their own adaptation mechanisms, the analyses were done twice, at the beginning of the experiment and 2 months later.

The design of this study was chosen in order to assess the possibilities of therapy with the use of the innovative molecule MSC-428 in patients with different tumor location, histology, various stages of chemotherapy and radiation therapy, as well as in patients who were not recommended to have standard antitumor therapy or the possibilities of standard antitumor therapy were found already invalid. The development of modern antitumor therapy is aimed at the existing genetic/biochemical changes of the tumor cell in a particular patient.

In oncology, a gradual transition from the treatment of the nosological form of the tumor to the treatment of the phenotypic and genetic changes which are present in the tumor cell in a particular patient, the so-called direction of Precision Oncology, is going to take place. In this connection, the importance of molecular, genetic and biochemical diagnostics of the existing abnormalities in tumor cells is increasing. Such kind of diagnostics is going to replace the morphological diagnosis. The nature of molecular genetic disorders determines the choice of drugs in the oncology clinic, and it helps to improve the efficiency of treatment.

Mercureid		Mercureid + Chemotherapy			control			
General condition of	number of patients		General condition of	number of patients		General condition of	number of patients	
WHO	abs.	%	WHO	abs.	%	WHO	abs.	%
I	9	5	1	6	3	I	12	7
I	27	14	II	45	26	II	48	30
Ш	120	63		102	59		66	40
M	36	18	IV	21	12	IV	38	23
Total	192	100	Total	174	100	Total	164	100

Information about patients	Mercureid	Mercureid + Chemotherapy	Standard Chemotherapy
Age (years)	28-74	29-75	32-81
Average age (years)	56,5	50,1	54
General condition (average) according to WHO	1,8	1,8	1,7
Diagnosis (number of patients)			
Non-small cell lung cancer	21	0	0
Small cell lung cancer	12	0	0
Prostate cancer	36	30	32
Breast cancer	42	39	42
Stomach cancer	27	27	34
Cervical cancer	0	24	28
Ovarian cancer	27	21	0
Cancer of the body of the uterus	9	18	28
Colon cancer	18	15	0
Total	192	174	164

Table 1: The characteristics of patients who were included into the research.

Statistical processing of the results was carried out using the program "STATISTICA", using parametric and nonparametric

methods of statistical packages. Among nonparametric methods, the Mann-Whitney-Wilcoxon (MWW) test was used, which is used for independent data fetch.

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### **Results and Discussion**

#### Immunological research

The basis of the pathogenesis of malignant tumors is immunological disorders which along with the damage of the cells genetic apparatus, are manifested by the dysfunction of the cell link of the immune system, distortion of the mechanisms of cell control and cells differentiation [26], immunological tolerance and impossibility of an effective immune response to the developing tumor.

The following American journals: PNAS (Proceedings of the National Academy of Sciences of the USA) and Cancer Discovery published the articles which proved the dominant role of the human immune system in the prognosis of breast cancer (BC). These discoveries were made while studying the interaction between T-helper (CD4), T-cytotoxic (CD8) lymphocytes and macrophages. The results of these studies bring to light the idea which cells of the immune system are the most important ones from the point of view of antitumor immunity and open the prospects for the search of immunological impact pathways.

# Dynamics of changes in lymphocytes CD3, CD4, CD8 and CD4/CD8 index

T-lymphocytes are morphologically indistinguishable from Blymphocytes. These cells are differentiated by the expression of marker molecules on their surface [27]. The common marker for all varieties of these T-lymphocytes that is absent in the other cells is the molecular complex TCR-CD3. The detection of CD3 - constant molecules, common for all T-lymphocyte varieties - is used to identify T-cells [28]. CD3 was first diagnosed in 1979 with the help of monoclonal antibodies. In the process of the antigen recognition by T-cells (more precisely, the complex of the antigenic peptide with the MHC molecule - Mayor Histocompatibility Complex), the additional molecules are involved along with the antigen-recognition receptor complex [29]. The most important ones are CD4 and CD8 co-receptors. The purpose of these molecules is primarily to increase the affinity of the receptor complex to the ligand by means of the additional binding of the co-receptors to MHC molecules (hence the definition of molecules as co-receptors) [30].

As the antigenic peptide can be presented to the T-cell in the composition of MHC molecules belonging to two different classes - I and II, two types of co-receptors - CD8 and CD4 - can participate in the recognition. CD8 molecule has an affinity for MHC-I, and CD4 molecule has an affinity for MHC-II [31]. On the mature T-cells, either CD4 or CD8 is expressed. Antigenic peptides in the composition of MHC-I molecules are recognized by CD8+ T cells, and the antigenic peptides in the composition of MHC-II molecules are recognized by CD4+ T cells. Co-receptors' binding increases 100 times the affinity of TCR to the antigenic complex [32].

Thus, the selection of T-lymphocytes in CD8+ and CD4+ is extremely important [32]. CD8+ T cells (T-killers) form a cytolytic molecular complex. It ensures the functioning of such T-cell as a cytotoxic T-lymphocyte. Most tumors are positive for HLA-I, but negative for HLA class II, and CD8 cells are capable of killing tumor cells by direct recognizing the peptide antigens presented by HLA-I molecules of malignant cells. CD8+ T cells are undoubtedly one of the most important subclasses of T-cells that effectively mediate antitumor immunity [33,34].

In CD4+ T-lymphocytes (T-helpers), the intracellular mechanisms necessary to perform the "helper" function, first of all - the ability to produce actively cytokines upon activation are formed. As a result, T-cells differentiate into functionally distinct subpopulations of cytotoxic and helper T-lymphocytes [35].

The study has shown that the patients have a decrease in lymphocyte count at the expense of cells with the phenotype of CD3+ and CD4+. It can be a result not only of tumor intoxication, but also the consequences of chemotherapy. Due to the low level of CD4+ lymphocytes, CD4/CD8 index was lowered. Mercureid intake did not have a stimulating effect on the indicators of patients with the initially normal number of T-lymphocytes and T-helpers. Mercureid intake promoted an increase in lymphocytes in percentage from  $16.5 \pm 2.4$  to  $23.9 \pm 3.2$  (+ 44.8%).

In Mercureid+ ChT group, it promoted an increase in lymphocytes in percentage from 17.2  $\pm$  3.6 to 24.2  $\pm$  2.7, respectively (+ 40.6%). In the control group, the changes did not have an obvious character - from 18.1  $\pm$ 4.1 to 19.4  $\pm$  3.6 (+7.1%).

The most significant changes were connected with an increase in number of CD3+ and CD8+, as well as normalization of the immunoregulatory index CD4/CD8 (Table 2).

	norm	Mercureid		Mercureid + Chemotherapy		control group	
index	%	before	after	before	after	before	after
Lymphocytes	19-37	16,5 ± 2,4	23,9 ± 3,2	17,2 ± 3,6	24,2 ± 2,7	18,1 ± 4,1	20,2 ± 3,6
T-lymph CD3	55-70	53,7 ± 5,3	65,2 ± 4,6	51,5 ± 7,4	59,8 ± 5,9	55,5 ± 6,3	57,8 ± 6,1
T-lymph CD4	40-60	37,7 ± 4,1	56,3 ± 5,3	38,9 ± 3,8	51,7 ± 6,2	43,9 ± 5,7	44,2 ± 3,8
T-lymph CD8	10-20	18,6 ± 3,4	12,3 ± 2,5	19,9 ± 3,7	16,2 ± 3,5	21,3 ± 4,1	20,2 ± 4,7
CD4/ CD8	2-4	1,9 ± 0,4	3,1 ± 0,7	2,1 ± 0,3	2,8 ± 0,4	1,8 ± 0,5	2,1 ± 0,6

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Table 2: Dynamics of change in lymphocytes CD3, CD4, CD8 and index CD4/CD8.

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The state of the immunoregulatory index.

In Mercureid group, the ratio changed from  $1.9 \pm 0.4$  to  $3.1 \pm 0.7$  (63.1% increase). In Mercureid+ ChT group, the ratio changed from 2,1 ± 0,3 to 2,8 ± 0,4 (33,3% increase). In the control group the ratio changed from 1,8 ± 0,5 to 2,1 ± 0,6 (19,2% increase).

# Dynamics of change in the number of lymphocytes with the phenotype CD16 (NK cells)

Natural killer cells, abbreviated NK are considered an important element of antitumor protection. A healthy person has the normal number of NK cells at least 170 in one microliter [36]. These cells take an active part in antiviral and antitumor protection. The specifics of NK cells actions are that they destroy the cells on which the expression of Class I HLA molecules is reduced [37].

On the surface of a natural killer, there are several important molecules on the presence or absence of which a cytotoxic response depends. One of such molecules is CD16 (a low affinity receptor for immunoglobulin G (IgG)). If there are G class antibodies on the surface of the tumor cell, it means that through these antibodies the natural killer, with the participation of CD16 molecule, joins the tumor cell [38]. Natural killers protect our body from tumor cells with a low expression of HLA molecules [39]. After the contact of the natural killer with the tumor cell, NK cell releases proteins - perforin, which get inserted into the membrane of the tumor cell and form a pore [40]. After that, the natural killer departs from the tumor cell, and through the pores formed by perforin, the intercellular fluid enters the tumor cell, the tumor cell swells and collapses [41].

On the surface of NK cells there are a lot of activation molecules. These are receptors for IFN-g, IL-2, IL-12, IL-15, IL-18 [42]. On the surface of all natural killers, there is a molecule called FAS ligand (CD178), which causes a programmed death of the target cell. The activation of NK cells IL-2, IL-12 leads to enhanced expression (CD 178) on NK cells [43]. The interaction of CD16 with IgG has a similar effect. Thus, the destruction of the tumor cell can also occur through the interaction of CD178 with the receptors of apoptosis on tumor cells. People who have a small number and/or low functional activity of NK cells more often undergo the risk of cancer development [44].

	Index	norm		after	
Nº	Natural Killers, CD16	%	before		
1	Mercureid	10 – 20%	8,9 ± 2,7	14,5 ± 3,1	
2	Mercureid + Chemotherapy	10 – 20%	10,4 ± 2,4	13,8 ± 3,4	
3	control group	10 – 20%	11,8 ± 3,2	12,2 ± 1,5	

\* p < 0,05

Dynamics of changes in lymphocytes, CD16 13.8 12.2 16 14 12 10 86 4 20 11.8 10,4 group Mercureid Mercureid group Chemotherapy Chemotherapy Mercureid + Mercureid + control control before after

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Table 3: Dynamics of change in lymphocytes CD16.

Taking into account the fact that only the dynamics of change in T-lymphocyte expression with CD16 marker are under consideration, these data do not reflect the complete picture connected with the restoration of cytolytic activity of cells. With this purpose, the percent of patients who got the restoration of killer activity of CD16 cells to the norm was investigated.

Nº	Index Natural Killers, CD16	before	after
1	Mercureid	27%	61%
2	Mercureid + Chemotherapy	29%	63%
3	control group	31%	34%







# Dynamics of change in the number of lymphocytes with the phenotype CD25, CD95

The researchers' interest to the process of programmed cell death - apoptosis has been increased lately [45]. Within the immune system, apoptosis is considered not only as a mechanism for negative selection of autoreactive clones in ontogeny, but also as a key mechanism for the regulation of T-cell homeostasis. The concept of apoptotic immunodeficiency is formulated and confirmed.

The increased availability of the cell 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 an apoptotic death process.

The recent studies show that the development of immunological imbalance, violation of lymphocytes activation, cytokine status (especially the use of chemotherapy drugs used in oncotherapy) is accompanied by a change in CD95 expression and is connected with abnormal apoptosis of immunocytes [46]. In this regard, the estimation of the number of CD95-expressing lymphocytes can be a valuable addition in the characterization of a subpopulation structure and a complex assessment of the immune status and can have a prognostic value [47,48].

In the study, the majority of patients (82%) had an increased expression of Fas receptor CD95+ and decreased to IL-2 (CD25), which is characteristic not only of the Th2 variant of the immune system response, but also of hyperproduction of pro-inflammatory cytokines [49,50]. CD95 (Fas or APO1) is a transmembrane glycoprotein belonging to the tumor necrosis factor family, and this molecule binding to Fas ligand leads to the induction of programmed cell death of CD4+, CD8+, CD16+ cells. It can be assumed that a high level of CD95+ and TNF $\alpha$  results in apoptotic death of activated lymphocytes.

In the group of patients who took Mercureid, we noted its effect on the increase in lymphocyte expression with CD25+ phenotype and decrease in CD95+, that correlates with an increase in the production of IL-2, cytokine which increases the cytolytic function of T-killers and NK cells, increases the production of perforin and interferon-gamma by these cells. Mercureid increases the production of IL-2 that increases the expression of MHC class I, improving the recognizability of tumor antigens. Interleukin-2 (IL-2) ensures the acceleration of the proliferation of T and B lymphocytes, the growth of the immune response to the T-dependent antigen, the restoration of the functional reserve of macrophages. Reducing the expression of CD95+ marker decreases the apoptotic readiness of lymphocytes. It increases the amount of CD4+, CD8+, CD16+ cells, compared to the control group.

norm Index %		Mercu	Mercureid		Mercureid + Chemotherapy		control group	
		before	after	before	after	before	after	
T-lymph CD25	10 – 18%	9,4 ± 2,1	15,3 ± 3,6	10,1 ± 2,5	13,2 ± 3,7	9,5 ± 1,9	11,8 ± 2,5	
T-lymph CD95	10 – 20%	24,7 ± 3,2	16,3 ± 3,4	23,5 ± 2,4	19,3 ± 3,7	25,1 ± 4,4	22,6 ± 2,7	
CD25/ CD95	0,5 – 1,8%	0,3 ± 0,04	0,9 ± 0,07	0,4 ± 0,03	0,6 ± 0,04	0,3 ± 0,05	0,5 ± 0,06	

\* p < 0,05



Table 5: Dynamics of change in lymphocytes CD25, CD9.

#### Phagocytosis

At the present time, the participation of phagocytosis in the processes of morphogenesis, elimination of cells after apoptosis and damaged after chemotherapy, formation of bacterial resistance is recommended. One of the most important functions of macrophages (along with chemotaxis, phagocytosis, secretion of biologically active substances) is the processing of antigen and its presentation to immunocompetent cells with the participation of proteins of the main histocompatibility complex (MHC) class II.

Tumor cells synthesize a factor that inhibits the migration of macrophages (MIF), which is an essential element of tumor growth. MIF in tumor growth performs simultaneously two important functions. Under the influence of MIF macrophages that have come to the tumor lose their mobility but preserve the ability to synthesize biologically active substances. MIF doesn't give macrophages a chance to transmit information about a detected tumor to other immunocompetent cells, allows the tumor to use an immobilized macrophage as a factory producing a large amount of plasminogen activator. Thanks to the activator of plasminogen, which is synthesized by macrophages, tumor cells acquire the ability to penetrate into the bloodstream and spread throughout the body. Thus, macrophages are attacked, both from the side of chemo- and radiotherapy, and the suppressive impact of the tumor itself.

In the current study, we revealed the stimulating effect of Mercureid on the phagocytic link of immunity providing an increase in the stability of the microorganism and in the growth of the antitumor potential. Determination of phagocytic activity of neutrophils by means of microscopic counting of phagocytic cells and phagocytic objects made it possible to specify a statistically reliable increase in the number of phagocytic cells. In Mercureid group, from  $1612 \pm 276$  to  $2934 \pm 301$  (82% increase). In Mercureid+ ChT group, from  $1759 \pm 391$  to  $2256 \pm 128$  (29% increase). In the control group, from  $1704 \pm 249$  to  $1809 \pm 261$  (6% increase).

N⁰	Index Phagocytosis	norm	before	after
1	Mercureid	1600 - 4000	1612 ± 276	2934 ± 301
2	Mercureid + Chemotherapy	1600 - 4000	1759 ± 391	2256 ± 128
3	control group	1600 - 4000	1704 ± 249	1809 ± 261

\* p < 0,05



Table 6: Dynamics of change in phagocytosis.

The most informative for phagocytic activity assessment is considered to be the absorption index, that is, the phagocytic number, the phagocytic number coefficient, which reflect the completeness of phagocytosis and the bactericidal index - the ability of the phagocyte to digest the captured microbe. The phagocytic index (PI) is the percentage of cells involved into phagocytosis from the total number of cells.

The phagocytic number (PN) is the average number of microbes absorbed by one neutrophil in the blood. This indicator characterizes the absorptive capacity of neutrophils.

Index	norm	Mercureid + contr Chemotherapy		Mercureid + Chemotherapy		control	group
	%	before	after	before	after	before	after
PI - 30	94,2 ± 1,5%	90,3 ± 2,4	94,1 ± 1,3	91,5 ± 2,3	93,8 ± 1,1	92,3 ± 2,7	93,1 ± 1,2
PI - 120	92,0 ± 2,5%	93,6 ± 1,9	92,3 ± 2,4	91,7 ± 2,5	92,8 ± 2,7	90,6 ± 2,5	91,6 ± 2,3
PN - 30	11,3 ± 1,0%	10,3 ± 2,4	11,5 ± 1,2	9,8 ± 1,9	11,7 ± 2,7	10,4 ± 1,9	11,2 ± 1,7
PN - 120	9,8 ± 1,0%	9,7 ± 1,5	10,2 ± 2,4	9,9 ± 2,3	10,5 ± 2,1	9,2 ± 1,7	9,6 ± 1,8

\* p < 0,05



**Citation**: Gusev SN., *et al.* "Results of Multitarget Therapy Anti-PD-1/PD-L1/CD19/CD25/CD38 with Application of MSC-428 Molecules in Patients with Different Oncopathology". *Acta Scientific Cancer Biology* 2.10 (2018): 03-22.

Results of Multitarget Therapy Anti-PD-1/PD-L1/CD19/CD25/CD38 with Application of MSC-428 Molecules in Patients with Different Oncopathology



Table 7: Dynamics of change in PI and PN (ability to capture and digest).

Both indicators are calculated on the swabs which are made after 30- and 90-minute (that is, a total of 120-minute) incubation, in other words, we mean PI-30 and PI-120, respectively PN-30 and PN-120.

As a result of the calculations, a decreased ability of cells readiness for phagocytosis is fixed. In Mercureid group, we have detected an increase from  $90.3 \pm 2.4$  to  $94.1 \pm 1.3$ . In Mercureid+ ChT group, from  $91.5 \pm 2.3$  to  $93.8 \pm 1.1$ . In the control group, only a slight increase from  $92.3 \pm 2.7$  to  $93.1 \pm 1.2$  has been detected.

The ability to digest antigens was initially reduced in all groups. After conducting the therapy, these indicators have changed: in Mercureid group, from  $10.3 \pm 2.4$  to  $11.5 \pm 1.2$ ; in Mercureid+ ChT group, from  $9.8 \pm 1.9$  to  $11.7 \pm 2.7$ ; in the control group, this indica-

tor hasn't come back to the normal state and it has changed only from  $10,4 \pm 1,9$  to  $11,2 \pm 1,7$ . Also the data that characterize the number of patients who, as a result of therapy, recovered phagocytic activity are of a great interest today.

These data are of a great significance, because phagocytosis is an important component of organism detoxification from the destroyed cells, it causes antibacterial resistance in patients compromised by chemo and radiotherapy. It participates in the system of antigen recognition and presentation of tumor proteins to immune cells. It causes activation signals of cytokines to mobilize an antitumor response of the body. Apparently, the activating stimuli for phagocytic cells were cytokines, in particular, IL-2 and interferon, whose production is enhanced by Mercureid action.

Citation: Gusev SN., et al. "Results of Multitarget Therapy Anti-PD-1/PD-L1/CD19/CD25/CD38 with Application of MSC-428 Molecules in Patients with Different Oncopathology". Acta Scientific Cancer Biology 2.10 (2018): 03-22.

No	Index	h - (	after	
NŸ	Phagocytosis	petore		
1	Mercureid	38%	78%	
2	Mercureid + Chemotherapy	41%	76%	
3	control group	35%	39%	



\* p < 0,05

**Table 8:** The number of patients whose phagocytic activity hasrecovered to the norm (%).

# Dynamics of change in the number of lymphocytes with the phenotype CD38

CD38 encodes a membrane protein involved in cell adhesion and catalyzes the formation of cyclic ADP-ribose. It is shown that in the patients' tumor foci there are cells in which stable expression of CD38 occurs. The previous studies showed that the level of CD38 increases with lung cancer, stomach cancer, and breast carcinoma. CD38 increases in breast carcinoma starting with the second stage of tumor growth and remains high in the subsequent stages, regardless of the metastases location.

The highest content of the antigen under study is observed with the combined histological form of the tumor; statistically reliable increased levels of CD38 remain both in the presence of single and multiple lesions. A high concentration of CD38 antigen is characteristic of tumors of any diameter.

There is a model of the participation of CD38 antigen in limiting the migration of mononuclear cells from the vascular bed to the tissue space, and, accordingly, to the spot of tumor localization. The presented model reflects one of the ways with the help of which the mechanisms for the escape of tumor cells from the immune system surveillance are formed. Thus, an increased level of CD38 antigen can be considered as one of the factors of tumor escape from the antitumor immune system response and the development of lymphocyte non-response to tumor antigens and the violation of the mechanism of PD-1/PD-L1 immune checkpoints. CD38 normalization occurs on the background of remission, thus it is advisable to use it as a monitoring prognostic indicator in the treatment of breast carcinoma].

N⁰	Index	norm %	before	after		
1	Mercureid	70 10 – 20%	25,3 ± 3,8	17,9 ± 2,6		
2	Mercureid + Chemotherapy	10 – 20%	24,6 ± 2,6	18,4 ± 2,5		
3	control group	10 – 20%	23,1 ± 1,7	21,2 ± 1,8		



\* p < 0,05

Table 9: Dynamics of change in lymphocytes CD38.

The change in the level of CD38 expression and activity is an adequate indicator of the intensity of the pathological process and the effectiveness of the therapy. In Mercureid group, there was a more obvious decrease in this indicator from  $25.3 \pm 3.8$  to  $17.9 \pm 2.6$ . In Mercureid+ ChT group, from  $24.6 \pm 2.6$  to  $18.4 \pm 2.5$ . In the control group, this indicator remained a little higher  $23.1 \pm 1.7$  to  $21.2 \pm 1.8$  than in the previous groups.

# Dynamics of change in the number of lymphocytes with the phenotype CD45

CD45, LCA, the common antigen of leukocytes belongs to the receptors protein tyrosine phosphatase family. This is transmembrane molecule with extracellular domain and cytoplasmic site consisting of two tandem catalytic phosphate domains.

In a number of researches, the interconnection between the immune reaction and the degree of the breast cancer spreading has been studied and a number of important regularities have been established with a decrease in the overall level of leukocyte infiltration (CD45+), the incidence of metastatic lesion of regional lymph nodes significantly increased. Detection of distant metastases negatively correlated with the overall level of immune response (CD45+). The obviously displaced general level of the local immune response, estimated by CD45, can serve as an independent factor of favorable prognosis in patients with breast cancer. The determination of CD45 showed that, with a mild reaction, the overall 5-year period of survival was  $61.4 \pm 9.3\%$ ; with a moderate reaction - 72,7 ± 7,0% and with the obviously displaced reaction -77,9 ± 5,3%.

**Citation:** Gusev SN., *et al.* "Results of Multitarget Therapy Anti-PD-1/PD-L1/CD19/CD25/CD38 with Application of MSC-428 Molecules in Patients with Different Oncopathology". *Acta Scientific Cancer Biology* 2.10 (2018): 03-22.

It should be noted that a change in the activation of CD45 induces the expression of transcriptional Bcl-6 inhibitor that regulates the expression of CTLA-4], which in its turn is a protein receptor that functions as an immune checkpoint, suppressing immune responses. CTLA4 acts as an "off" switch when it is bound to CD80 or CD86 on the surface of antigen-presenting cells.

Nº	Index	norm	boforo	after	
	CD45	%	belore		
1	Mercureid	12 – 24%	11,7 ± 2,1	18,7 ± 2,4	
2	Mercureid + Chemotherapy	12 – 24%	12,4 ± 3,2	15,3 ± 2,6	
3	control group	12 – 24%	10,7 ± 1,9	12,9 ± 1,9	



#### \* p < 0,05

Table 10: Dynamics of change in lymphocytes CD45.

In Mercureid group, there was a more intense increase in CD45 protein tyrosine phosphatase from  $11.7 \pm 2.1$  to  $18.7 \pm 2.4$ . In Mercureid+ ChT group, from  $12.4 \pm 3.2$  to  $15.3 \pm 2.6$ . In the control group, this indicator remained at the lower limit of the norm  $10.7 \pm 1.9$  to  $12.9 \pm 1.9$ .

# Dynamics of change in the number of lymphocytes with the phenotype CD19

CD19 is the membrane antigen of B cells. The molecular weight is 120 kDa. It belongs to the immunoglobulin superfamily]. B-lymphocytes process tumor antigens and get activated. In activated B-lymphocytes, the antigen is present on the membrane in combination with MHC class II protein. The mature T-helper cell binds to the activated B-lymphocyte. It results in the allocation of interleukin-2 by T-helper. Under the influence of interleukin-2 the B-cell is divided and differentiated, turning into a plasma cell. A mature plasma cell secretes antigen-specific immunoglobulins (antibodies). Tumor-specific antibodies bind to tumor antigens. But the tumor cell has a specific feature: it can lose its surface antigens. The man's background level of complement is insufficient for the development of antibody-dependent lysis of the tumor cell. The antigen-antibody complex leaves the tumor cell earlier than the activation and polymerization of the complement start. The antitumor antibodies which are under formation process and circulating immune complexes aggravate the development of the disease. They block the antigens of tumor cells and T-killer receptors, protecting the tumor cell from a cytolytic attack.

During the research, various changes in B-cell expression were fixed.

In Mercureid group, at the beginning of the study, a higher level of B-lymphocytes with CD19 marker was noted. It was  $17.8 \pm 2.6$ . Later it decreased to  $13.8 \pm 1.9$ . In Mercureid+ ChT group, initially 42% of patients had this index lowered. It was  $10.1 \pm 2.5$  (on average) but it had a tendency to increase to  $12.8 \pm 2.6$ . It could be due to the consequences of chemotherapy, which had a negative impact on the system of humoral immunity. In the control group, 39% of patients had a low level of CD19 cells. During the examination period this index remained practically at the same level. It could also be due to the conducted chemotherapy courses.

N⁰	Index	norm	hefore	after	
	CD19	%	Delore		
1	Mercureid	6 – 15%	17,8 ± 2,4	13,8 ± 1,9	
2	Mercureid + Chemotherapy	6 – 15%	10,1 ± 2,3	12,8 ± 2,1	
3	control group	6 – 15%	8,7 ± 1,8	9,3 ± 2,3	



\* p < 0,05

#### Table 11: Dynamics of change in lymphocytes CD19.

Due to the multidirectional changes in the number of CD19 cells (due to the presence of chronic diseases, duration of chemotherapy, surgical treatment, antibiotic therapy), the dynamics of recovery to the physiological norm, the number of CD19 cells is of great importance.

#### Dynamics of change of immunoglobulins Classes A, M, G

Humoral immunity factors play an unequal role in antitumor immunity. First, antibodies can cause antigenic modulation of tumors, that is, antigens sloughing and their endocytosis and the appearance of antigens with new features. Second, due to the specific characteristics of tumor antigens, namely their low density on the cell membrane, not all classes of immunoglobulin play a role as antitumor factors. For example, they can promote tumor growth,

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Nº	Index	before	after	
	CD19	norm	norm	
1	Mercureid	35%	71%	
2	Mercureid + Chemotherapy	31%	68%	
3	control group	29%	32%	



#### \* p < 0,05

**Table 12:** The number of patients with CD19 norm recovery (%).

it means that they have an effect of immunological enhancement. Immunological enhancement connected with IgG is determined by the fact that binding of antigenic determinants of tumor antigens is not accompanied by the activation of the complement system.

Because of the low density of antigens of the tumor cell membrane between Fc fragments of immunoglobulins, a critical distance is not reached, that is considered a necessary condition for the activation of the first component of the complement system. As a result, the tumor cell remains viable. The screened antigenic determinants are not recognized by immune killers and NK cells. If, with tumor antigens, IgM interacts, it means that complement-dependent cytolysis is possible. This is due to the fact that one molecule of IgM is sufficient to activate the complement system. The specific structure of IgM is constructed in such a way that between the Fc fragments of the subunits, there is a distance necessary to bind the first component of the complement system to form an immune complex. But for this action it is necessary that at least 2 IgM subunits bind to antigenic determinants. In such a way, IgM performs a function directed against tumors. However, it should be taken into account that IgM concentration is significantly lower than IgG. Besides, the main amount of IgM is found only in the blood, because it does not penetrate through the vascular wall.

During the examination, the patients who suffer chronic diseases of the gastrointestinal mucosa (gastritis, colitis, cholecystopancreatitis) had a higher IgA level, probably as the body's response to inflammation.

As a result of Mercureid intake, normalization of IgA indicators was fixed. Taking the individual analyses the patients, who had an initially lowered indicator, got the results with its increase to the norm, and the patients who had an initially high indicator, got the results with its decrease to the norm. It can be connected with the anti-inflammatory properties of Mercureid, aimed at inflammation reducing. A part of patients participating in the research at the first examination showed a low amount of IgM and IgG that can be connected with both genetic characteristics and transient immunodeficiency in the form of humoral immune disorders.

Indov	norm	Merc	ureid	Mercureid + Chemotherapy		control group	
index	mg/мl	before	after	before	after	before	after
lgA (mg/мl)	1,2 – 2,0	2,91 ± 0,89	1,97 ± 0,45	2,72 ± 0,89	2,18 ± 0,75	2,87 ± 0,38	2,81 ± 0,79
lgM (mg/мl)	0,9 – 1,2	0,67 ± 0,12	0,92 ± 0,18	0,69 ± 0,19	0,91 ± 0,27	0,71 ± 0,16	0,82 ± 0,19
lgG (mg/мl)	9 – 18	14,6 ± 2,34	16,07 ± 2,57	15,07 ± 2,57	16,3 ± 3,51	12,51 ± 1,67	10,7 ± 2,71

\* p < 0,05







**Table 13:** Dynamics of change of immunoglobulins.

Because the levels of immunoglobulin were of an individual character and depended on the activity of the patients' chronic diseases (either increased or decreased) at the beginning of the study, it was decided to fill in the table the percentage of those patients whose immunoglobulin levels corresponded to the physiological norm.

	Mercureid		Mercureid + Chemotherapy		control group	
Index	before	after	before	after	before	after
	norm	norm	norm	norm	norm	norm
lgA (mg/мl)	32%	61%	29%	58%	34%	37%
lgM (mg/мl)	31%	67%	34%	61%	32%	39%
lgG (mg/мl)	64%	76%	65%	72%	65%	67%



**Citation:** Gusev SN., *et al.* "Results of Multitarget Therapy Anti-PD-1/PD-L1/CD19/CD25/CD38 with Application of MSC-428 Molecules in Patients with Different Oncopathology". *Acta Scientific Cancer Biology* 2.10 (2018): 03-22.

\* p < 0,05



Table 14: Dynamics of norm recovery of immunoglobulin Class A, M, G (%).

# Dynamics of change in the number of lymphocytes with the phenotype CD54

ICAM-1 or CD54 is a single-chain glycoprotein with a molecular weight of 55 kDa. This membrane protein belongs to the immunoglobulin superfamily and is an adhesion molecule. Adhesion molecules are involved in the progressing of the tumor and its metastasis. An important factor of tumor angiogenesis is the protein - a vascular endothelial growth factor (VEGF), which stimulates the proliferation and migration of endothelial cells of nearby vessels. The expression of VEGF in malignant tumors is combined with the increased metastatic activity and decreased survival.

The directional migration of endothelial cells is the key to tumor neoangiogenesis. The ICAM-1 molecule is involved in the regulation of endothelial cell mobility. ICAM-1 controls the migration of endothelial cells, promoting directional movement, by means of developing cellular polarity and influencing on the activity of lamellipodia.

When the cell moves, the bundle of actin filaments periodically pushes out the lamellopodia and microchips at the front leading edge of the cell and stretches the cell cortex, polarizing the cell that helps it move forward. Lamellopodia (from Latin "Lamina" a thin leaf, "under" - leg) are the protrusion of the actin protein cytoskeleton on the front edge of the cell. During polarization, an actin moves the lamella forward and in such a way acts as a driving mechanism for the cells during chemotaxis. Lamellopodium is actually an engine that drives the cell forward in the process of migration. Movable cells are extremely sensitive adhesion detectors. Lamellopodia which are produced by the endothelial cell move towards the most adhesive part of the substrate, i.e they are attracted by ICAM-1 molecules. If the signal is absent, they stop. In this regard, the important role of adhesion molecules in providing neovascularization of the tumor gets obvious. If the VEGF protein only signals about the beginning of vascular growth, it means that the performers who attract and control the direction of endotheliocyte movement are ICAM-1 molecules.

The dynamics of change in the expression of CD54 molecules (ICAM-1) in different groups of patients are presented below. The obtained data correspond to the positive result of the treatment as the tumor size decrease or even a complete regression of the tumor process in case of a decrease in the expression of CD54 molecules (ICAM-1).

N⁰	Index	norm	boforo	after	
	CD54	%	DEIDIE		
1	Mercureid	10 – 20%	24,3 ± 2,3	15,8 ± 2,9	
2	Mercureid + Chemotherapy	10 – 20%	23,5 ± 2,7	18,8 ± 3,1	
3	control group	10 – 20%	25,1 ± 2,8	22,8 ± 1,6	



\* p < 0,05

Table 15: Dynamics of change in CD54 (ICAM-1).

It can be supposed that Mercureid molecules violate the stability of the tubulin cytoskeleton of endothelial cells, as a result the ability of endothelial cells to proliferate, move, interact intercellularly become lower. In the end, the gaps, through which the blood plasma leaves, are formed between the endothelial cells.

The local viscosity of the blood increases and when the platelets come in contact with the exposed basal membrane, the coagulation process with the formation of tissue necrosis is triggered.

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The results of using the new MSC-428 molecule in the complex therapy in 530 patients showed the formation of a stable antitumor patient response. It is targeted for 4 target proteins, such as: PD-1/PD-L1, CD19, CD25, CD38. It should be noted that their activity recovery is largely predetermined by the normalization of other surface antigens of lymphocytes, such as: CD3, CD4, CD8, CD16, CD45, CD95, phagocyte system, immunoglobulin class A, M, G. In our opinion, only complex actions ensure the formation of complete, effective and safe immunotherapy in patients.

#### Conclusions

- 1. At the beginning of the study, patients had lymphopenia. In Mercureid group, there was an increase in the number of lymphocytes from  $16.5 \pm 2.4\%$  to  $23.9 \pm 3.2\%$  (44.8% increase). In Mercureid+ ChT group, the changes were the following: from  $17.2 \pm 3.6$  to  $24.2 \pm 2.7$  (40.6% increase). In the control group, the changes were not so obvious 18.1  $\pm$  4.1 to  $20.2 \pm 3.6$  (11.1% increase). The norm is 19 37%.
- 2. CD4+ T-lymphocytes are required to perform antigen-presenting function, cytokine production and activation of the cytotoxic immune response. In Mercureid group, before the treatment this indicator was  $37.7 \pm 4.1\%$ , after the treatment it became  $56.3 \pm 5.3\%$  (49.3% increase). In Mercureid+ ChT group, before the treatment this indicator was 38,9  $\pm$  3,8%, after the treatment it became  $51.7 \pm 6.2\%$  (32.9% increase). In the control group, before the treatment it was  $43.9 \pm 5.7\%$ , after the treatment  $44.2 \pm 3.8\%$  (it remained almost at the same level). The norm is 40-60%.
- 3. CD8+ T-cells (T-killers) form a cytolytic molecular complex that ensures this cell functioning as a cytotoxic T-lymphocyte. A reduced number of CD4+ cells result in the development of CD8+ cell tolerance to antigen and lymphocyte anergy. The dynamics of CD8+ cells level normalization in Mercureid group was 18.6  $\pm$  3.4% before the treatment, and 12.3  $\pm$  2.5% after the treatment. In Mercureid+ ChT group, this indicator was 19.9  $\pm$  3.7% before the treatment, and 16.2  $\pm$  3.5% after the treatment. In the control group, 21.3  $\pm$  4.1% before the treatment, and 20.2  $\pm$  4.7% after the treatment. The norm is 10-20%.
- 4. Corresponding to the changes in the amounts of CD4+ and CD8+ cells, the immunoregulatory index CD4/CD8 has changed. Initially, it was reduced due to the cells imbalance. After the treatment in Mercureid group, it normalized from  $1.9 \pm 0.4$  to  $3.1 \pm 0.74$ . In Mercureid+ ChT group, it had the indicators from  $2.1 \pm 0.3$  to  $2.8 \pm 0.4$ . In the control group the indicators were from  $1.8 \pm 0.5$  to  $2.1 \pm 0.6$ . The norm is 2-4. These changes are statistically significant p<0.04 and 0.017, respectively.
- 5. The number and activity of NK cells were initially decreased in Mercureid group and had the indicator  $8.9 \pm 2.7\%$ . After the treatment the indicator changed into:  $14.5 \pm 3.1\%$ (62% increase). In Mercureid+ ChT group, before the treat-

ment the indicator was  $10.4 \pm 2.4\%$ , after the treatment - 13.8  $\pm$  3.4% (32.6% increase). In the control group, before the treatment the indicator was  $11.8 \pm 3.2\%$ , after the treatment - 12.2  $\pm$  1.5% (3.4% increase). The norm is 10 - 20%.

- 6. Before the treatment, in Mercureid group, 27% of patients had the normal level of NK cells and 61% of patients got recovery to the normal functioning during the treatment. In Mercureid+ ChT group, 29% of patients had the normal level of NK cells and 63% of patients got recovery to the normal functioning. In the control group, 31% of patients had the normal level of NK cells before the treatment and at the end of the study, 34% of patients had recovery to the normal functioning.
- 7. CD25 is a marker of early activation, receptor for IL-2. It is expressed in developing and activated lymphocytes. In Mercureid group, there were changes from  $9.4\% \pm 2.1\%$  to  $15.3\% \pm 3.6\%$  (62.7% increase). Mercureid+ ChT group demonstrated the indicators from  $10.1 \pm 2.5\%$  to  $13.2\% \pm 3.7\%$  (30.6% increase). In the control group, respectively, the indicators were from  $9.5 \pm 1.9\%$  to  $11.8 \pm 2.5\%$  (24.2% increase). The norm 10 18%.
- 8. CD95 expressing lymphocytes were increased in all groups. But, the more intensive recovery occurred in Mercureid group: from 24.7  $\pm$  3.2% to 16.3  $\pm$  3.4% (34% decrease). Mercureid+ ChT group had the following changes, 23.5  $\pm$  2.4% at the beginning of the study, 19.3  $\pm$  3.7% (18% decrease) after the treatment. In the control group, the indicators were the following: from 25.1  $\pm$  4.4% at the beginning, and 22.6  $\pm$  .7% at the end (10% decrease). The norm is 10-20%.
- 9. The phagocyte system is important in terms of elimination of the cells that died after chemotherapy, antigen processing and its presentation to the immunocompetent cells with proteins of the main histocompatibility system (MHC) class II. The phagocyte system is affected toxically not only by palliative chemotherapy (PCT), but also by the tumor itself. In Mercureid group, the recovery of patients' phagocytic activity from 38% to 78%, (p < 0.05) occurred statistically reliable. In Mercureid+ ChT group: from 41% to 76%. In the control group, the recovery of patients' phagocytic activity was insignificant from 35% to 39%, respectively, p < 0.05.
- 10. An increased number of CD38 antigens on the cell surface helps the tumor to escape from the immune response and promotes apoptotic death of immunocytes.

Some researchers suggest using it as a prognostic factor for the therapy success.

In Mercureid group, the indicator decreased from  $25.3 \pm 3.8\%$  to  $17.9 \pm 2.6\%$  (29.6% decrease). In Mercureid+ ChT group, the indicator decreased from 24.6  $\pm$  2.6% to 18.4  $\pm$  2.5% (25.2% decrease). In the control group, it decreased from 23,1  $\pm$  1,7% to 21,2  $\pm$  1,8% (8,3% decrease). The norm is 10-20%.

11. The high expression of CD45 marker correlates with the patient's 5-year survival. In Mercureid group, there was a more intensive increase in the general leukocyte marker CD45 from  $11.7 \pm 2.1$  to  $18.7 \pm 2.4$ . In Mercureid+ ChT group: from  $12,4 \pm$ 

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3,2 to  $15,3 \pm 2,6$ . In the control group, this indicator remained at the lower limit of the norm  $10.7 \pm 1.9$  to  $12.9 \pm 1.9$ . The purpose of Mercury is to provide a higher survival rate.

12. In Mercureid group, at the beginning of the study, there was an increased level of B-lymphocytes (CD19), which amounted to  $17.8 \pm 2.6\%$  and then decreased to  $13.8 \pm 1.9\%$ . In Mercure-ide+ ChT group, this indicator was reduced in 42% of patients and averaged  $10.1 \pm 2.5\%$ , having a tendency to increase up to  $12.8 \pm 2.6\%$ .

We can suppose that it is connected with the consequences of chemotherapy, which had a negative impact on the system of humoral immunity. In the control group, a low level of CD19 cells was fixed in 39% of patients. It was  $8.7 \pm 1.8\%$  at the beginning and  $9.3 \pm 2.3\%$  at the end. During the examination period it remained practically at the same level. It could also be connected with the chemotherapy courses. The norm of CD19 is 6-15%.

- 13. The study of the level of immunoglobulin class A, M, G has shown the ability of the drug to reduce the increased values of IgA that can probably be connected with the anti-inflammatory properties of the drug. Mercureid increased the initially lower values of IgM, which the complement-dependent cytolysis of tumor cells is capable of providing. The level of IgG, on average, was normal in all groups.
- 14. The important data about the role of CD54 markers (ICAM-1) in tumor angiogenesis have been obtained. ICAM-1 molecule is involved in the regulation of endothelial cell mobility. ICAM-1 controls the migration of endothelial cells, promoting the directed movement, by means of developing cellular polarity and affecting the activity of lamellipodia. The increased expression of these markers promotes the intensive formation of vessels that feed the tumor. Reduction decreases the activity of tumor angiogenesis. In Mercureid group, the dynamics of decrease in CD54 adhesion molecules (ICAM-1) was as follows, before therapy 24.3  $\pm$  2.3%, after 15.8  $\pm$  2.9% (decrease by 35%). In Mercureid+ ChT group, before therapy to 23.5  $\pm$  2.7%, after 18.8  $\pm$  3.1% (decrease by 20%). In the control group, before therapy 25.1  $\pm$  2.8%, after 22.8  $\pm$  1.6% (decrease by 9%).
- 15. In general, using pathomorphological evaluation criteria, including therapeutic pathomorphosis of III-IV degree, the efficiency of the treatment was the following: 67.3% in Mercureid group, 61.7% in Mercureid+ chT group and 54.8% in the control group.

The use of Mercureid in complex therapy provides the formation of a stable antitumor response of the patient. It is targeted for 4 target proteins, such as: PD-1/PD-L1, CD19, CD25, CD38. It should be noted that their activity recovery is largely predetermined by the normalization of other surface antigens of lymphocytes, such as: CD3, CD4, CD8, CD16, CD45, CD95, phagocyte system, immunoglobulin class A, M, G. In our opinion, only complex actions ensure the formation of complete, effective and safe immunotherapy in patients. The use of the drug in immunotherapy in patients with oncopathology:

- Prevents the apoptotic death of immunocytes, induced both by the tumor, and the consequences of special treatment.
- Normalizes the number of lymphocytes with the phenotype CD3, CD4, CD8.
- Increases the killer activity of NK cells (CD16), CD8.
- Reduces the hyperactivity of proinflammatory cytokines.
- Recover the phagocyte system, damaged by chemotherapy and radiation therapy.
- Reduces the expression of CD38, preventing the effect of immune failure to respond to a tumor.
- Activates CD45 that increases the chances of a patient's survival.
- Reduces the expression of CD54 (ICAM-1) preventing tumor angiogenesis.
- Produces a complex effect on PD-1/PD-L1/CD19/CD25/ CD38 that increases the ability of the patient's immune system to kill tumor cells more effectively.

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