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REVIEWS

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MACROPHAGES — A PERSPECTIVE TARGET FOR ANTINEOPLASTIC IMMUNOTHERAPY

O.M. Karaman, A.V. Ivanchenko, V.F. Chekhun*

R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology, NAS of Ukraine, Kyiv 03022, Ukraine

The review discusses the data on the functional/phenotypic M1/M2 types of macrophages and their chimeric forms, the molecular mechanisms of polarization of these cells, and their role in the development of malignant tumors. Information on the prognostic value of the presence (density and location) of M1/M2 cells in tumor tissue is analyzed. Our own results evidence on the necessity of determination of the functional/phenotypic state of M1/M2 macrophages from different biological niches in the dynamics of tumor growth, in particular in terms of NO level and arginase activity.

***Key Words*: M1/M2 macrophages, plasticity, polarization, malignant tumors.**

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Among the important cells of the immune system, which in addition to effector functions exert also regulatory ones, resulting in the interconnection, regulation and functioning of cells of nonspecific and specific immunity, including antitumor immunity, are the cells of monocyte-macrophage origin — macrophages. Tissue macrophages and blood monocytes make up the system of mononuclear phagocytes and are the major cells of the innate immunity system. Macrophages are the first line of defense and provide an instant response of the body to the penetration of a foreign agent, and also are involved in triggering the response of the acquired immunity system.

According to modern data [1], macrophages are defined as a group of terminally differentiated cells of the mononuclear phagocytic system, which play an important role in tissue homeostasis, inflammation and protection against infectious pathogens, etc. The versatility of the functions of these cells is due to the expression of various surface membrane receptors, in particular: receptors of phagocytosis (lectin receptors, Fc-receptors, scavenger receptors); adhesion receptors (selectins, integrins); receptors participating in the presentation of Ag (major class 2 histocompatibility complex, MHC II); activation receptors (cytokine receptors, chemokine receptors); monocyte activation receptor CD14 [2].

Macrophages have a unique feature — plasticity, through which functional reprogramming of these cells is possible. There are two main types of functional state of macrophages: M1 and M2 cells. The functions and role of these two types of macrophages have been sufficiently studied in different pathological conditions (trauma, infectious processes, malignant growth, etc.). Regarding the role of macrophages in the development of malignant tumors, it is known that M1 macrophages exert an immunostimulatory and antitumor effect, and M2 has an immunosuppressive and protumoral effect. Moreover, it was shown that the change of macrophages from M1 in M2 is a reversible process [3] and depends on the microenvironment signals.

In the tumor structure, where a significant portion of the cells of the immune system are represented by tumor-associated macrophages (TAM) [4], which can be of both M1 and M2 types, tumor cells and tumor microenvironment direct the polarization of macrophages into M2 type. The prevalence of the latter in the TAM subpopulation is associated with poor clinical prognosis [5, 6]. It should be noted that TAM have attracted the attention of researchers long ago as promising targets for immunotherapy of patients with malignant tumors. Early works on the development of immunotherapeutic approaches using TAM were focused on strategies for blocking M2 protumoral activity. But later the focus of research has changed for a searching the opportunities to reprogram M2 into M1 type [7].

Today, new information is constantly emerging about the molecular mechanisms of macrophage polarization, macrophage subtypes and/or transient states and their role in the formation and direction of immune responses in various pathological conditions, including the formation and progression of malignancies. Such an array of data requires analysis and generalization. Therefore, this review analyzes the current data on macrophage types, their properties, polarization mechanisms, and their role in tumor growth. Understanding the features of macrophage polarization can be the basis for developing strategies for managed reprogramming of these cells, which will expand the array of antitumor immunotherapeutic approaches and increase their effectiveness.

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*Correspondence: E-mail: kmolga1977@gmail.com

Abbreviations used: CSF2Rα – colony stimulating factor receptors; EC – Ehrlich carcinoma; GM-CSF – granulocyte-macrophage colony stimulating factor; IFNγ – interferon γ; iNOS – inducible NO synthetase; IRF – interferon regulatory factors; HIF – hypoxia-induced factors; LLC – Lewis lung carcinoma; LPS – lipopolysaccharide; M-CSF – macrophage colony-stimulating factor; NK cells – natural killer cells; NO – nitric oxide; NSCLC – non-small cell lung cancer; PPARs – peroxisome proliferator-activated receptors; ROS – reactive oxygen species; TAM – tumor-associated macrophages; TGFβ – transforming growth factor beta; Th 1 – T helper 1 lymphocytes; TLRs – Toll-like receptors; TNFα – tumor necrosis factor α.

As is known, the cellular composition of tumor tissue is heterogeneous and extremely diverse and represents a set of both tumor cells heterogeneous in their biological properties and other types of non-tumor cells that form a tumor microenvironment. More details on the cellular composition of the tumor and its microenvironment can be found in the reviews [8–10]. At the same time, one of the key cells of the immune system — macrophages that regulate/determine the interaction of tumor cells with microenvironment during all stages of tumor development and also make up a considerable part of the immune population represented in the structure of the tumor — continue to be actively investigated. Tumor infiltrating macrophages are combined under general name "tumor-associated macrophages" (TAM). They were first discovered and described in 1992 by Stein *et al.* [11] as alternative to IL-4-activated $CD206^+$ cells. There are two ways of macrophage activation: the classical one, in which macrophages are polarized into M1 type and exhibit antitumor properties, and an alternative with subsequent formation of M2 type cells with protumoral action. Let's analyze in more detail the basic characteristics of M1 and M2 cells, as well as the mechanisms of their polarization.

THE MAIN CHARACTERISTICS OF M1 AND M2 CELLS, AND THE MECHANISMS OF THEIR POLARIZATION

Precursors of tissue M1/M2 macrophages are myeloid $CD34^+$ bone marrow cells, from which circulating blood monocytes are formed through successive stages of differentiation. The latter, after extravasation through the endothelium into the tissue, can there differentiate into macrophages or transdifferentiate into endothelial cells [12].

In the tissue, depending on the microenvironmental signals, macrophages, due to their plasticity, can acquire different functional phenotypes: from initiation of proinflammatory reactions to termination of inflammation and tissue repair. Thus, traditionally there are known *classically activated macrophages M1* and *alternatively activated macrophages M2* [13]. Later, information emerged about *innately activated macrophages* that result from the binding of pathogen-associated molecular patterns to pattern recognition receptors, including Toll-like receptors (TLRs). Such macrophages possess some properties of M1 cells: production of proinflammatory cytokines and microbicidal activity [14]. Also distinguished are the so-called *M2-like macrophages*, which are characterized by some properties of M2 macrophages [15]. It should be noted that the results of intensive studies of molecular mechanisms of polarization of macrophages into M1/M2 type, different sources of production of these cells, different methodological approaches to the identification of M1/M2 cells have led to the need to revise the conventional classification of macrophages. Thus, in 2014, Murray *et al.* [16] published a paper in which the authors propose to classify macrophages based on their sources of excretion, activation signals, and generally accepted investigated markers of macrophage activation, which will allow more correctly to divide macrophages into separate subtypes among M1 and M2, and compare the data from different labs.

Generalized and systematized data on the characteristics of the major macrophage types (M1 and M2), taking into account current data, are presented in the Table.

Table. Main characteristics of classically activated (proinflammatory) M1 and alternatively activated (anti-inflammatory) M2 macrophages

Characteristic	Macrophages type	
	classically activated (proinflammatory) M1	alternatively activated (anti-inflammatory) M2
Activation stimuli	Microbial products (e.g., LPS)	IL-4
	IFNγ	IL-13
	GM-CSF	IL-10
		IL-33
		M-CSF
Receptor profile	CD11c+	CD36+
	CD68+	CD68+
	CD80/CD86+	CD163+
	CD25+	CD204+
	IL-1R1	CD206+
	TLR	CD301+
Chemokines	CXCL1-3	CCL2
	CXCL9	CCL17
	CXCL10	CCL22
	CCL5	CCL24
Cytokines	IL-1	IL-10
	IL-6	PGE2
	IL-12	TGFβ
	IL-23	
	TNFα	
Production	ROS	Arginase-1
	NO	
Metabolic profile	Anaerobic glycolysis	Aerobic glycolysis
	Blocking of iron secretion (inhibition of ferroportin, activation of H-ferritin)	Enhanced iron secretion (activation of ferroportin, inhibition of H-ferritin)

Note: GM-CSF – granulocyte-monocyte colony-stimulating factor; IFNγ – interferon γ; LPS – lipopolysaccharides; M-CSF – macrophage colony-stimulating factor; NO – nitric oxide; ROS – reactive oxygen species; TNFα – tumor necrosis factor α; TGFβ – transforming growth factor β.

M1-polarized macrophages ($CD68^+$, $CD11c^+$, $CD80/CD86^+$, $CD25^+$ cells) are activated due to the action of interferon γ (IFNγ), microbial products (e.g., lipopolysaccharides (LPS)) or granulocyte-monocyte colony-stimulating factor (GM-CSF) (*classic activation pathway*). These cells produce mainly proinflammatory cytokines (IL-1, IL-6, IL-12, IL-23 and tumor necrosis factor α (TNFα)), resulting in activation of T helper 1 lymphocytes (Th1) and natural killer cells (NK cells). Here, the latter (both Th1 and NK cells) contribute to the polarization of macrophages in M1 type through the production of IFNγ. M1 macrophages are characterized by pronounced synthesis of CXCL1-3, CXCL9, CXCL10 and CCL5 chemokines, which enhances cytotoxic T-cell activation and resistance to intracellular pathogens and tumors. The receptor repertoire of M1 macrophages includes IL-1R1, TLR, CD80 and CD86 — T cell activation coregulators. These cells have a high ability to represent the antigen, as well as to produce reactive oxygen species (ROS) and nitric oxide (NO), whose increased production

is due to increased expression of inducible NO synthetase (iNOS) [17, 18]. M1 macrophages are characterized by certain features of metabolism of iron, folate and glucose. In particular, these cells block the secretion of iron (this process is called sequestration) by inhibiting ferroportin and activating H-ferritin, which enhances bacteriostatic effects [15]. Also, for the functioning of M1 the rapid release of energy is required which is provided by anaerobic glycolysis [19]. Due to these characteristics of M1, macrophages are considered to be cells with a distinct microbicidal and antitumor effect.

M2 polarized macrophages ($CD68^+$, $CD163^+$, $CD204^+$, $CD206^+$ cells) are activated by IL-4, IL-13, IL-10, IL-33 and monocyte colony-stimulating factor/CSF-1 (*alternative activation pathway*). Producer cells of these lymphokines are Th2 lymphocytes, basophils, cells of innate immunity, which creates the microenvironment necessary for alternatively activated macrophages. M2 cells are usually characterized by the ability to produce a large number of anti-inflammatory cytokines (especially IL-10) and a small number of pro-inflammatory cytokines (in particular, IL-12), as well as COX-2, prostaglandin E2 (PGE2), chemokines CCL2, CCL17, CCL22 and CCL24. These chemokines attract Th2 type lymphocytes and regulatory T cells (Treg) that promote M2 polarization. M2 cells are characterized by high expression of mannose receptors (CD206), galactose residues and N-acetylglucosamine (CD301), and "scavenger receptors" (CD36, CD163, stabilin 1). M2 secretes arginase-1 and can also produce extracellular matrix components and enzymes for its modeling, in particular fibronectin, tenascin C, matrix metalloproteinases [19–22]. M2 macrophages, like M1, have certain peculiarities in iron and glucose metabolism: increased iron secretion through activation of ferroportin and inhibition of H-ferritin and hemooxygenase, which leads to increased proliferation of cells in tissue that infiltrate M2 [15]. Glucose metabolism in M2 cells occurs through its aerobic glycolysis, which provides these cells with long-term energy supply for their functioning. In particular, M2 macrophages create an anti-inflammatory environment, provide processes for the regeneration and healing of wounds, and in the presence of a tumor, promote its progression [21, 23–25]. Under chronic pathology, M2 macrophages play a pro-fibrotic role, secreting immunosuppressive factors, including transforming growth factor β (TGFβ) and platelet growth factor [25–27].

M2 macrophages are further classified into subtypes: M2a, M2b, M2c and M2d, which are characterized by a unique gene expression profile, but equally high secretion of IL-10 and IL-1 receptor antagonist (IL-1RA) and low secretion of IL-12 [28, 29]. The formation of M2 macrophage subtypes depends on the activation stimulus: due to the action of IL-4 (or, in some cases, IL-13), the M2a subtype is formed, the effect of IL-1 or immune complexes in combination with LPS leads to polarization in M2b, the effect of IL-10 and glucocorticoids activate the M2c, IL-6, and macrophage colony-stimulating factor (M-CSF) phenotype leads to the formation of M2d (TAM) cells [29, 30].

Due to the simultaneous effects of immune complexes with LPS and/or IL-1, glucocorticoids, TGFβ and IL-10, or only IL-10, macrophages can polarize into M2-like macrophages. The latter are characterized by the expression of $CD163^+$, $CD206^+$, as well as the ability to produce large amounts of IL-10, pentraxin 3, chemokines CCL1, CCL16, CCL18, PGE2 and a small amount of IL-12, TNF, IL-6, IL-1β and chemokine CCL3.

Note that transient states of activated macrophages (such as M2-like macrophages or macrophages with the phenotype of M1 cells with simultaneous expression of M2-type genes) are detected at different stages of the development of the organism (eg, in placenta and embryo) and at pathological conditions (obesity) and bacterial infections, helminth invasion, rheumatoid arthritis, cancer) [15, 31]. In the study [31], the formation of chimeric M2-like macrophages, which responded to the stimulation of TLR2 ligands (but not TLR4 ligands) by secreting proinflammatory cytokines and reducing anti-inflammatory activity without significantly changing their M2 cell surface marker profile, was reported [31]. In this study, macrophages M2 were obtained by exposure of peripheral blood monocytes of patients with rheumatoid arthritis to M-CSF monocytes for 8 days. As ligands for TLR2 and TLR4 there were used Pam3CysSerLys4 (Pam3) or LPS or IFNγ/LPS, respectively. The profile of surface markers for M1/M2 was: $CD14^+$, $CD163^+$, $CD206^+$, $CD86^+$, $CD80^+$.

MACROPHAGE POLARIZATION MECHANISMS AND THE CONCEPT OF "PLASTICITY"

Let's consider in more detail what molecular mechanisms are involved in the polarization of macrophages in the M1 or M2 phenotypic/functional state. According to the literature analyzed, molecular modulators of M1/M2 polarization involve different inflammatory modulators (TLRs), signaling molecules (interferon regulatory factors (IRFs), signal transducers and STAT transcriptional activators), transcription factors (NF-κB, Kruppel-like factor, peroxisome proliferator-activated receptors (PPARs)), hypoxia factors (hypoxia-induced factors (HIF) 1α, HIF2α), micro-RNA (miRNA), etc. Central to the direction of macrophage polarization is the STAT/IRF signaling pathway. For the activation of the STAT/IRF signaling pathway the primary stimulating signals from TLRs or the corresponding cytokine receptors (IFNα/βR, IFNγR, IL-10R, IL-4Rα, IL-13Rα1) and colony stimulating factor receptors (CSF2Rα) are important [32–34].

LPS and other microbial ligands activate TLR4, which is a known signal for the formation of the macrophage M1 phenotype. Moreover, the TLR4/TLR2 ratio is much higher in M1 macrophages compared to M2 cells [35, 36]. Through activated TLRs, the signal is transmitted to IRFs: activation of IRF3 and IRF5 triggers signaling cascades of M1 polarization, and IRF4 — M2 polarization of macrophages [17, 37,

38]. Activation of IFN type I and II (IFNα/βR, IFNγR) receptors results in the polarization of M1 macrophages through the activation of STAT1, and the IL-4 and IL-13 (IL-4Rα, IL-13Rα1) receptors, and also IL-10 (IL-10R) — to polarize M2 through activation of STAT6 and STAT3 respectively. The signal from the CSF2Rα (under the influence of the GM-CSF) leads to the polarization of macrophages in M1 through the activation of STAT5 [35]. That is, polarized M1 macrophages are characterized by activation of the IRF3 and STAT1/IRF5 and STAT5 signaling pathways, and polarized M2 macrophages — by activation of STAT6/IRF4 and STAT3. Note that LPS activation of TLR4 results in the expressed secretion of cytokines by macrophages (IL-1β, IL-6, IL-12, TNF and IFNβ), chemokines (CCL2, CXCL10, CXCL11), as well as the expression of MHC molecules; upon stimulation of macrophages by GM-CSF, a somewhat less pronounced secretion of proinflammatory cytokines is observed [33].

A key role in macrophage polarization is played by proteins of the NF-κB family, which control the activity of the genes required for this process [17]. The most important representatives of this family of proteins p65 and p50. The formation of p65/p50 heterodimer activates the genes of acute inflammatory mediators (TNFα, IL-1β, IL-6, IL-12 (p40), IFNγ, CXCL10 and NOS2 [20, 25]), which are characteristic of M1 macrophages. The p50/p50 homodimer exhibits an antagonistic effect on the p65/p50 complex (its binding with the same promoters blocks the expression of the latter), so that macrophages acquire features of M2-type cells [36, 39].

Macrophage polarization also involves miRNAs, which are short non-coding RNAs of about 21–23 nucleotides in length and can regulate gene expression at the posttranscriptional level [39–43]. miRNAs play a key role in a variety of biological processes, such as embryogenesis, differentiation, inflammation, viral infections, and carcinogenesis. Up to date the importance of miRNAs in the development of the immune system and the formation of the immune response has been demonstrated. It has been shown that miRNAs are involved in the regulation of immunity, including the development and differentiation of immune cells, the production of antibodies, and the release of inflammatory mediators [44]. Regarding the role of miRNAs in macrophage polarization, it is shown that M1 type is characterized by increased expression of miRNA-125a, miRNA-125b miRNA-127, miRNA-155, miRNA-378, and for M2 — miRNA let-7c, miRNA-9, miRNA-21, miRNA-146, miRNA147, miRNA-187, miRNA-195 [16, 37, 45, 46]. It should be noted that in differential analysis of expression of miRNAs in M1 and M2 macrophages, only 8 from 109 studied miRNAs were selected, for which more than 2-fold increase or decrease of expression was observed. In M1 macrophages compared with M2, more than 2-fold increase in expression of miR-181a, miR-155-5p, miR-204-5p, miR-451 ($p < 0.05$), and decreased expression of miR-125-5p, miR-146a-3p, miR-143-3p and miR-145-5p (fold change <-2, $p < 0.05$) was observed (qRT-PCR data). These data indicate the necessity to investigate a number of miRNAs when studying their role in macrophage polarization [46]. The following will provide information on the role of only some of the miRNAs mentioned above that are involved in the polarization of macrophages and their targets.

The activity of miR-125b is to inhibit the IRF4, which is a negative regulator of proinflammatory macrophage activation. miRNA-125b is associated with improved antigen presentation, increased T-cell activation, and tumor destruction [47]. The study [48] showed that overexpression of miR-125a in macrophages leads to increased secretion of proinflammatory cytokines and NO production by these cells, indicating M1 polarization; regulation of expression of miR-125a/miR-99b is mediated by Notch signaling. Previously, another group of researchers [49] showed that Notch signaling (via the Notch1-NIC intracellular domain) not only directs TAM differentiation into M1 cells but also counteracts their protumoral function via miR-125a. Transfection of murine macrophages with miR-125a mimetics led to an increase in their phagocytic activity against L1210 cells *in vitro*, and the simultaneous subcutaneous transplantation of such transfected macrophages with Lewis lung carcinoma (LLC) cells inhibited the growth of the latter by immune microenvironment. There was observed the decrease of $CD11b^+Ly6G^+$ macrophage count in the tumor and spleen of animals and simultaneous increase of $CD3^+CD8^+$ lymphocyte counts in tumor tissue and peripheral lymph nodes. The authors conclude that miRNA-125a may be a target for reprogramming M2 macrophages in tumor microenvironment in M1 cells and restoring their antitumor activity.

For the formation of M1, and a significantly enhanced production of proinflammatory cytokines by these cells, an increased expression of miR-127, which is induced upon activation of TLRs, is required. The absence of miR-127 shifts the polarization in the M2 phenotype [50]. Another key molecule in M1/M2 macrophage polarization is miRNA-155, the target of which is the C/EBPβ transcription factor (characteristic for M2 macrophages). The overexpression of miR-155 has been shown to lead to the formation of the M1 phenotype and its decrease — of M2 [51, 52]. The target of miR-155 may also be IL-13Rα1, the inhibition of which blocks the IL-13-induced M2 phenotype [53].

For the formation of M2 macrophages (unlike M1) are important other types of miRNAs. In particular, it was shown [54] that let-7c miRNA is expressed in M2 macrophages at a higher level than in M1; moreover, the LPS-induced stimulation of M2 cells reduced the expression of this miRNA. Overexpression of miRNA let-7c led to a decreased expression of the M1 phenotype with simultaneous polarization into the M2 phenotype and *vice versa*. An transcription factor C/EBP-d important for the formation of inflammatory

responses has been found to be the target for let-7c. In addition, let-7c has been shown to regulate bactericidal and phagocytic activity of macrophages. miRNA-146a modulates macrophage polarization through the Notch 1 signaling pathway or the PPARγ receptor. Its overexpression is observed in M2 macrophages and leads to increased expression of M2-marker genes (Arg1, CD206). In M1 macrophages, overexpression of miRNA-146a significantly reduces the production of proinflammatory cytokines and iNOS [45]. The role of miRNA-195 has also been shown regarding the direction of macrophage polarization [55]. In particular, M2c-type macrophages (derived from human peripheral blood monocytes after IL-10 stimulation) an increased level of this miRNA compared to M1 macrophages (derived from human peripheral blood monocytes after LPS stimulation) was observed. In LPS- and IFN-γ -stimulated macrophages of the TNR-1 line an overexpression of miRNA195 was shown to result in a decreased levels of TLR2, phosphorylated forms of p54 JNK, p46 JNK and p38 MAPK kinases and IL-1β, IL-6 and TNFα in supernatants. That is, miRNA195 inhibits the proinflammatory polarization of M1 type macrophages.

The study [56] showed that macrophage polarization can also be controlled by the PPARγ/miRNA223 regulatory axis. Alternative macrophage activation of miRNA223 targets has been shown to be Rasa1 and Nfat5 [55], and in classical macrophage activation — the pro-inflammatory regulator Pknox1, which, as noted above, mediates such activation [57].

In addition to the transcription factors IRF/STAT and miRNA, macrophage polarization can be affected by hypoxia through HIF [58, 59]. Thus, Th1 cytokines are able to induce HIF1α by triggering M1 activation, whereas Th2 cytokines activate HIF2α, which promotes M2 macrophage activation. It should be noted that HIF1 and HIF2 are able to regulate (activate or suppress) NO synthesis, which in turn also determines the functional activity of macrophages [60].

Regardless of the primary polarization and the degree of differentiation, macrophages may change their phenotypic/functional state in response to microenvironment signals. The importance of the influence of microenvironment for the formation of phenotypes of macrophages is given in numerous reviews by Mantovani [21, 61–63]. The ability of macrophages to change their phenotypic/functional state is termed *plasticity*, and the process itself — reprogramming. Plasticity ensures that macrophages perform their functions in both physiological and pathological processes. It should be noted that macrophages are highly plastic cells that can be reprogrammed with different stimuli [31, 64, 65]. For example, it was shown that peritoneal macrophages isolated from mice with model MFC gastric tumors identified as M2, were reprogrammed into M1 type macrophages after *in vitro* treatment with IL-12 and IFNγ. This led to the emergence of antitumor activity in treated macrophages, in contrast to the cells that did not undergo such treatment and were isolated from intact or tumor-bearing animals [36, 39].

MACROPHAGE POLARIZATION IN THE TUMOR LESION AND THEIR ROLE IN CANCER

In the tumor lesion, the microenvironment promotes TAM polarization in M2 type, and according to data [29, 30] — in M2d cells. One of the mechanisms of reprogramming is the effect of tumor cells on proteins of the NF-kB family. In particular, in the work [36] it was shown that tumor cells can cause disruption of p65 protein synthesis in macrophages and stimulate the accumulation of the p50/p50 inhibitory complex that promotes the reprogramming of M1 cells into M2. During the development of the tumor, new macrophages are involved in the growth of its lesion, which are reprogrammed into M2 cells. TAM2 promotes tumor progression and metastasis through direct immunosuppressive action and/or through the involvement of other types of immunosuppressive cells (in particular, Treg, myeloid suppressive cells, etc.) [4, 66], as well as by modeling the extracellular matrix of tumor (utilizing the factors secretion similarly to tissue repair), or by modeling of suppressor genes (or their products) in tumor cells using miRNAs through isolation of miRNAs-containing exosomes. Thus, in the work [62] it was shown that macrophages derived from colorectal cancer M2 tissue produced exosomes containing miR-21-5p and miR-155-5p. As a result of the addition of such exosomes to tumor cells, a decrease in BRG1 expression was observed, which the authors identify as a key factor in metastasis of colorectal cancer cells, since its reduced level was registered in metastatic cells.

In the study [67], using *in vivo* model (MK2 KO transgenic mice) and *in vitro* models (human cell lines: U-937 monocytes and HUVEC endothelial cells) it was also demonstrated that among TAM exactly M2 polarized cells contribute to tumor progression. In particular, in MK2 KO mice characterized by tissue-specific expression of p38/MAPKAP kinase 2 (MK2) with chemically induced rectal tumor, tumor progression and active tumor neoangiogenesis was observed compared with animals with blocked MK2 function. That is, inhibition of p38/MAPKAP kinase 2 (MK2) in M2 macrophages leads to a shift in their polarization toward M1, as well as to disruption of M2-induced angiogenesis.

Today it is convincingly shown that the presence of TAM of type M2, but not M1, is considered a poor prognostic marker in patients with solid malignant tumors. In this case, different markers are used to determine the amount of TAM: total macrophagal — CD68, M1 markers — CD11c, CD86, M2 markers — CD163, CD204, CD206 [68–70]. In particular, in the work [71], in the study of tumor tissue of 62 patients with renal cell carcinoma, it was shown that the presence of $CD163^+$ TAMs is associated with poor clinical prognosis (in univariate analysis). It was also

found that direct *in vitro* coculturing of the obtained macrophages with tumor cells (of Caki-1, ACHN, 786-0 and MAMIYA lines) resulted in stable activation of Stat3 in the latter due to the binding of membrane macrophage colony-stimulating factor (mM-CSF) with M-CSF receptor on TAM.

Another group of researchers using immunohistochemical analysis assessed infiltration of hepatocarcinoma tissue of 80 patients with $CD68^+$ TAM, including $CD11c^+$ inflammatory (M1) and $CD206^+$ immunosuppressive (M2) macrophages, as well as the density of the present TAM [72]. There was no correlation between the presence of $CD68^+$ macrophages in the tumor and the overall survival rate, and no correlation between the CD68, CD11c and CD206 TAM density in tumor tissue and survival. However, correlations were found between the presence of $CD11c^+$ (positive correlation) or $CD206^+$ (negative correlation) macrophages and overall survival. Using a multivariate Cox regression analysis, it was determined that the presence of $CD11c^+$ and $CD206^+$ TAMs are independent prognostic factors ($p < 0.001$, $p = 0.031$, respectively). Regarding the expression of the investigated markers as prognostic indices, the presence of $CD68^+$ cells, as well as the low expression of CD11c correlated only with the stage of the disease. In contrast, high CD206 expression correlated with patients' age, tumor size, vascularization, metastatic status, and the stage of the disease.

In the work [73], the phenotype (CD68, CD86, and CD206) and the TAM density in hepatocellular carcinoma tissue of 253 patients were also investigated. The presence of $CD68^+$ TAM was found to be unrelated to clinical and pathological characteristics and prognosis. Low levels of $CD86^+$ tumor infiltration by macrophages and high $CD206^+$ cell infiltration rates correlated with tumor aggression rates, including multiple tumors and stage III–IV, and were associated with low overall survival ($p = 0.027$ and $p = 0.024$, respectively). The authors noted that in patients with hepatocellular carcinoma the result of simultaneous analysis of the level of $CD86^+$ and $CD206^+$ cells was more significant for the assessment of overall survival ($p = 0.011$) than the evaluation of each index alone. In addition, the $CD86^+/CD206^+$ TAMs ratio was predictive of overall survival ($p = 0.002$) in α-fetoprotein-negative patients. The researchers suggested that the combined determination of CD86 and CD206 TAMs may be a promising prognostic biomarker for hepatocellular carcinoma.

The results of the analysis of the content of M1/M2 macrophages in tumors of 80 patients with non-small cell lung cancer (NSCLC) of stages I–III were presented in [74]. Macrophages were detected by immunohistochemical double staining using the following markers: CD68/iNOS for M1, CD68/CD163 for M2. In addition to the number and polarization of macrophages, their location was taken into account: tumor islets or tumor stroma. The authors noted that infiltration by macrophages was observed mainly in the stroma of the tumor than in its islets. Moreover, in the latter, M2 prevailed among the infiltrating macrophages. It was found that indices of the presence of M1 in tumor islets, as well as the simultaneous presence of M2 in tumor islets and stroma, are independent predictors of survival of patients with NSCLC: high infiltration of tumor islets by M1 macrophages is associated with an increase in overall survival rate ($p < 0.05$); high infiltration of tumor islets and stroma by M2 macrophages — with its decrease ($p < 0.05$) [74].

In the study [75], it was first determined that angiopoietin-like protein 2 (Angptl2) promotes M2 polarization of TAM, which in turn leads to the progression of the tumor process. Clinical material of patients with NSCLC (stages I–IV) showed that Angptl2 expression on tumor cells was aberrantly increased and correlated positively with the amount of TAM ($r = 0.5848$; $p < 0.01$), tumor size ($p = 0.022$), with the disease stage ($p = 0.037$) and a decrease in the 5-year survival rate of patients. In *in vitro* experiments (co-culturing of macrophages with tumor cells of the HMLC line; stimulation of macrophages with recombinant Angptl2) and *in vivo* (nude BALB/c mice with H1299 tumors overexpressing Angptl2), it was determined that Angptl2 stimulates polarization of TAM in M2 via activation of p65-NF-kB pathway, and Angptl2-stimulated TAMs also enhance NSCLC cell proliferation, invasion, and migration. The authors suggest that Angptl2 protein may be an effective target for reprogramming TAMs in NSCLC tumors, the use of which will improve the effectiveness of treatment of patients with NSCLC.

So, there is a fair amount of compelling information on the importance of identifying the TAM phenotype for prognosis, as well as the choice of further therapeutic tactics. On the other hand, it seems appropriate to change the tumor microenvironment, in particular, reprogramming of TAMs and changing their properties from protumoral to antitumoral. However, information about the so-called "transient forms" of macrophages must be taken into account. The issue of correct identification of M1 and M2 macrophages in *in vivo* and *in vitro* studies is addressed in the work [16]. This is important because the activation and polarization of these cells is associated with significant shifts in gene expression (hundreds of genes) depending on the activation stimuli, none of which definitively determines the formation of a specific subpopulation or activation state of macrophages. That is, to differentiate macrophages into M1/M2 only by CD-markers, as used in the evaluation of lymphocyte subpopulations, is not a sufficient condition. The authors of the article point out the need in the process of M1/M2 identification to take into account the source of macrophages, activation signals, and indices of their activity (arginase activity, production of NO and ROS, gene expression of pro- or anti-inflammatory cytokines, etc.). In our opinion, estimation of NO level and arginase activity appears to be quite informative in the differential determination of M1/M2 polarization of macrophages,

since it is known that in M1 and M2 macrophages the metabolism of arginine is high [76]. In particular, the iNOS enzyme expressed in M1 macrophages metabolizes arginine to NO and citrulline. NO can be metabolized for the subsequent release of reactive nitrogen species, whereas citrulline can be used for efficient NO synthesis through the citrulline-NO cycle. Arginase, which is expressed in M2 macrophages, hydrolyzes arginine to ornithine and urea. The arginase pathway limits the availability of arginine for NO synthesis, and ornithine itself can further participate in polyamine and proline synthesis pathways that are important for cell proliferation and tissue repair. Both metabolic pathways of arginine cross-inhibit each other at the level of the corresponding breakdown products of arginine, which, in our opinion, leads to a more accurate determination of M1 or M2 polarization status.

We have conducted preliminary studies to assess polarization of macrophages isolated from various organs and tissues of C57Bl mice (lungs, spleen, peritoneal washes) with LLC or BALB/c mice with Ehrlich carcinoma (EC) (spleen, peritoneal washes) cancer, by NO and arginase activity. Macrophages derived from the spleen or peritoneal cavity in the LCC growth dynamics were found to polarize toward type 2 cells; moreover, on the 28-th day of tumor growth, the highest arginase activity was exhibited by macrophages isolated from the peritoneal cavity. Pulmonary macrophages were characterized by proinflammatory M1 type throughout the observation period (periods of primary tumor growth and metastases). Given that the ratio of NO production to the arginase activity of macrophages derived from the spleen and the peritoneal cavity was characterized by a clear stable tendency to decrease during these periods, it can be assumed that reprogramming of the lung macrophages into an anti-inflammatory type could be observed in later observation terms [77].

In mice with EC, on the 22-nd day of tumor growth macrophages derived from the peritoneal cavity or from the spleen were characterized by proinflammatory M1 type. Moreover, the ratio of NO production to arginase activity (NO/arginase) in mice with EC was 23.3 and 24.2 against 19.2 and 21.9 in intact animals. On the 28-th day of observation (terminal stage of the tumor process), spleen macrophages of mice with EC were characterized by higher NO production and reduced arginase activity than peritoneal macrophages, i.e. functional polarization of spleen derived macrophages could be determined as antitumor one.

Our data substantiate the importance and further feasibility of the study of the functional/phenotypic status of macrophages from different biological niches in the early stages of malignancy. The results of such studies may be optimally matched timing for the influence on the monocyte/macrophage component for reprogramming macrophages into anticancer M1 cells and triggering a cascade of reactions that will stop the progression of the disease.

So, the analysis of current data on the molecular mechanisms of macrophage polarization allowed to identify not only M1 and M2 types, but also M1 or M2-like chimeric cell forms, confirming the diversity (heterogeneity) of the macrophage subpopulation, as well as the need for a multifaceted approach for macrophage identification (activation stimuli, expression of CD markers, production of cytokines, chemokines, NO, arginase activity, expression of certain genes). This is especially important when using TAM as prognostic factors in the course of the tumor process, as well as the selection of strategic approaches to reprogramming of macrophages into a proinflammatory type or changing their microenvironment, which will contribute to the formation of M1 type. This approach in immunotherapy will be an additional tool for improving the effectiveness of treatment of patients with malignant tumors.

REFERENCES

1. **Petty AJ, Yang Y.** Tumor-associated macrophages: implications in cancer immunotherapy. Immunotherapy 2017; **9**: 289–302.

2. **Kzhyshkowska JuG, Gratchev AN.** Monocyte and macrophage markers for diagnostics of immunopathologies. Pathogenesis 2012; **10**: 14–9 (in Russian).

3. **Sawa-Wejksza K, Kandefer-Szerszen M.** Tumor-associated macrophages as target for antitumor therapy. Arch Immunol Ther Exp 2018; **66**: 97–111.

4. **Tang X, Mo C, Wang Y.** Anti-tumour strategies aiming target tumour-associated macrophages. Immunology 2013; **138** : 93–104.

5. **Qian BZ, Pollard JW.** Macrophage diversity enhances tumor progression and metastasis. Cell 2010; **141**: 39–51.

6. **Kovaleva OV, Efremov GD, Mikhaylenko DS.** Role of tumor-associated macrophages in renal cell carcinoma pathogenesis. Cancer Urol 2017; **1**: 20–6 (in Russian).

7. **Rhee I.** Diverse macrophages polarization in tumor microenvironment. Arch Pharm Res 2016; **39** (11): 1588–96.

8. **Chekhun VF, Sherban SD, Savtsova ZD.** Tumor heterogeneity — dynamical state. Onkologiya 2012; **5**: 4–12 (in Ukrainian).

9. **Berezhnaya NM.** Role of immune system cells in tumor microenvironment. I. Cells and cytokines — the components of inflammation. Onkologiya 2009; **1**: 6–17 (in Russian).

10. **Berezhnaya NM.** Role of immune system cells in tumor microenvironment. II. Interaction of the immune system cells with other microenvironment components. Onkologiya 2009; **2**: 86–93 (in Russian).

11. **Stein M, Keshav S, Harris N, Gordon S.** Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation. J Exp Med 1992; **176**: 287–92.

12. **Das A**, **Sinha M**, **Datta S**, ***et al.*** Monocyte and macrophage plasticity in tissue repair and regeneration. Am J Pathol 2015; **185**: 2596–605.

13. **Mills CD, Kincaid K, Alt JM, *et al.*** M-1/M-2 macrophages and theTh1/Th2 paradigm. J Immunol 2000; **164**: 6166–73.

14. **Rodrıguez-Prados J-C, Traves P, Cuenca J, *et al.*** Substrate fate in activated macrophages: a comparison between innate, classic, and alternative activation. J Immunol 2010; **185**: 605–14.

15. **Biswas SK, Mantovani A.** Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. Nat Immunol 2010; **11**: 889–96.

16. **Murray PJ, Allen JE, Biswas SK, *et al.*** Macrophage activation and polarization: nomenclature and experimental guidelines. Immunity 2014; **41**: 14–20.

17. **Gordon S, Martinez FO.** Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; **8**: 958–69.

18. **Tacke F, Zimmermann HW.** Macrophage heterogeneity in liver injury and fibrosis. J Hepatol 2014; **60**: 1090–6.

19. **Sarbaeva NN, Ponomareva JV, Milyakova MN.** Macrophages: diversity of phenotypes and functions, interaction with foreign materials. Genes Cells 2016; **11**: 9–17 (in Russian).

20. **Schaer DJ, Boretti FS, Hongegger A.** Molecular cloning and characterization of the mouse CD163 homologue, a highly glucocorticoid-inducible member of the scavenger receptor cysteine-rich family. Immunogenetics 2001; **53**: 170–7.

21. **Sakhno LV, Shevela EYa, Chernykh ER.** Phenotypic and functional characteristics of the alternative activated macrophages: potential use in regenerative medicine. Immunologiya 2015; **36**: 242–6 (in Russian).

22. **Gratchev A, Kzhyshkowska J, Utikal J, Goerdt S.** Interleukin-4 and dexamethasone counterregulate extracellular matrix remodelling and phagocytosis in type-2 macrophages. Scand J Immunol 2005; **61**: 10–7.

23. **Sakhno LV, Tikhonova MA, Shevela EY, *et al.*** Phenotypic and functional features of human M2-like macrophages. Bull SB RANS 2014; **34**: 18–24 (in Russian).

24. **Koh TJ, Di Pietro LA.** Inflammation and wound healing: the role of the macrophage. Expert Rev Mol Med 2011; **13**: 23.

25. **Sun YY, Li XF, Meng XM, *et al.*** Macrophage phenotype in liver injury and repair. Scand J Immunol 2017; **85**: 166–74.

26. **Zhang F, Wang H, Wang X, *et al.*** TGF-β induces M2-like macrophage polarization via SNAIL-mediated suppression of a pro-inflammatory phenotype. Oncotarget 2016; **7**: 52294–306.

27. **Gratchev A, Kzhyshkowska J, Kannookadan S.** Activation of a TGF-beta-specific multistep gene expression program in mature macrophages requires glucocorticoid-mediated surface expression of TGF beta receptor II. J Immunol 2008; **180**: 6553–65.

28. **Hao NB, Lü MH, Fan YH, *et al.*** Macrophages in tumor microenvironments and the progression of tumors. Clin Dev Immunol 2012; **2012**: 948098.

29. **Mantovani A, Sozzani S, Locati M, *et al.*** Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002; **23**: 549–55.

30. **Wang Q, Ni H, Lan L, *et al.*** Fra-1 protooncogene regulates IL-6 expression in macrophages and promotes the generation of M2d macrophages. Cell Res 2010; **20**: 701–12.

31. **Lilian Q, Edveena H, Tobias M, *et al.*** TLR2 stimulation impairs anti-inflammatory activity of M2-like macrophages, generating a chimeric M1/M2 phenotype. Arthritis Res Ther 2017; **19**: 245.

32. **Nan W, Hongwei L, Ke Z**. Molecular mechanisms that influence the macrophage M1–M2 polarization balance. Immunobiology 2012; **217**: 1233–40.

33. **Essandoh K, Li Y, Huo J, Fan GC.** MiRNA-mediated macrophage polarization and its potential role in the regulation of inflammatory response. Shock 2016; **46**: 122–31.

34. **Sauer RS, Hacke lD, Morsche lL, *et al.*** Toll like receptor (TLR)-4 as a regulator of peripheral endogenous opioid-mediated analgesia in inflammation. Mol Pain 2014; **10**: 10.

35. **Nan W, Hongwei L, Ke Z.** Molecular mechanisms that influence the macrophage M1–M2 polarization balance. Immunology 2014; **614**: 1–9.

36. **Hao L, Xiaoling W, Nie G, *et al.*** Macrophage functional phenotype can be consecutively and reversibly shifted to adapt to microenvironmental changes. Int J Clin Exp Med 2015; **8**: 3044–53.

37. **Wang N, Liang H, Zen K.** Molecular mechanisms that influence the macrophage M1-M2 polarization balance. Front Immunol 2014; **5**: 614.

38. **Calin GA, Croce CM.** MicroRNA-cancer connection: the beginning of a new tale. Cancer Res 2006; **66**: 7390–4.

39. **Jiang J, Lee EJ, Gusev Y, Schmittgen TD.** Real-time expression profiling of microRNA precursors in human cancer cell lines. Nucl Acids Res 2005; **33**: 5394–403.

40. **Lee EJ, Baek M, Gusev Y, *et al.*** Systematic evaluation of microRNA processing patterns in tissues, cells lines and tumors. RNA 2008; **14**: 35–42.

41. **Acunzo M, Romano G, Wernicke D, Croce CM.** MicroRNA and cancer — A brief overview. Adv Biol Regul 2014; **57**: 1–9.

42. **Sethupathy P.** Illuminating microRNA transcription from the epigenome. Curr Genom 2013; **14**: 68–77.

43. **Li Z, Chao TC, Chang KY, *et al.*** The long noncoding RNA THRIL regulates TNFα expression through its interaction with hnRNPL. Proc Natl Acad Sci USA 2014; **111**: 1002–7.

44. **Bi Y, Liu G, Yang R.** MicroRNAs: novel regulators during the immune response. J Cell Physiol 2009; **218**: 467–72.

45. **Huang C, Liu XJ, Qun Zhou, *et al.*** MiR-146a modulates macrophage polarization by inhibiting Notch1 pathway in RAW264.7 macrophages. Int Immunopharmacol 2016; **32**: 46–54.

46. **Yingying Z, Mengying Z, Min Z, *et al.*** Expression profiles of miRNAs in polarized macrophages. Int J Mol Med 2013; **31**: 797–802.

47. **Graff JW, Dickson AM, Clay G, *et al.*** Identifying functional microRNAs in macrophages with polarized phenotypes. J Biol Chem 2012; **287**: 21816–25.

48. **Qian L, Xia H, Qiao Y, *et al.*** The Notch signal mediates macrophage polarization by regulating miR-125a/miR-99b expression. Jing Chen Pages 2019; **29**: 833–43.

49. **Zhao JL, Huang F, He F, *et al.*** Forced activation of notch in macrophages represses tumor growth by upregulating miR-125a and disabling tumor-associated macrophages. Cancer Res 2016; **76**: 1403–15.

50. **Ying H, Kang Y, Zhang H, *et al.*** MiR-127 Modulates macrophage polarization and promotes lung inflammation and injury by activating the JNK pathway. Immunol 2015; **194**: 1239–51.

51. **Xing C, Yuan Y, Ningzhu L, *et al.*** Re-polarization of tumor-associated macrophages to pro-inflammatory M1 macrophages by microRNA-155. J Mol Cell Biol 2012; **4**: 341–3.

52. **Zhang P, Wang H, Luo X, *et al.*** MicroRNA-155 inhibits polarization of macrophages to M2-type and suppresses choroidal neovascularization. Inflammation 2018; **41**: 143–53.

53. **Martinez-Nunez RT, Louafi F, Sanchez-Elsner T**. The interleukin 13 (IL-13) pathway in human macrophages is modulated by microRNA-155 via direct targeting of interleukin 13 receptor alpha1 (IL13Ralpha1). J Biol Chem 2011; **286**: 1786–94.

54. **Banerjee S, Xie N, Cui H, *et al.*** MicroRNA let-7c regulates macrophage. J Immunol 2013; **190**: 6542–9.

55. **Bras JP, Silva AM, Calin GA, *et al.*** miR-195 inhibits macrophages pro-inflammatory profile and impacts

the crosstalk with smooth muscle cells. PLoS One 2017; **12**: e0188530.

56. **Ying W, Tseng A, Chang RC, *et al.*** MicroRNA-223 is a crucial mediator of PPARγ-regulated alternative macrophage activation. J Clin Invest 2015; **125**: 4149–59.

57. **Zhuang G, Meng C, Guo X, *et al.*** A novel regulator of macrophage activation: miR-223 in obesity-associated adipose tissue inflammation. Circulation 2012; **125**: 2892–903.

58. **Nefedova NA, Davidova SU.** The role of vascular endothelial growth factor and hypoxia-inducible factor in tumor`s angiogenesis. Modern Problems Sci Edu 2015; (3): 51 (in Russian).

59. **He C, Carter AB.** The metabolic prospective and redox regulation of macrophage polarization. J Clin Cell Immunol 2015; **6**: 371–7.

60. **Lan J, Sun L, Xu F, *et al.*** M2 macrophage-derived exosomes promote cell migration and invasion in colon cancer. Cancer Res 2019; **79**: 146–58.

61. **Sica A, Mantovani A.** Macrophage plasticity and polarization: *in vivo* veritas. J Clin Invest 2012; **122**: 787–95.

62. **Mantovani A, Allavena P, Sica A, Balkwill F.** Cancer-related inflammation. Nature 2008; **454**: 436–44.

63. **Balkwill F, Charles KA, Mantovani A**. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 2005; **7**: 211–7.

64. **Monastyrskaya EA, Lyamina SV, Malyshev IYu.** Ml and M2 phenotypes of activated macrophages and their role in immune response and pathology. Pathogenesis 2008; **6**: 31–9 (in Russian).

65. **Mosser DM, Edwards JP.** Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; **8**: 958–69.

66. **Skivka LM, Gorbik GV, Fedorchuk OG, Pozur VV.** Tumor-associated macrophages in the perspective of developing methods of cancer target therapy. Cytology Genetics (Ukr) 2009; **43**: 71–82 (in Ukrainian).

67. **Suarez-Lopeza L, Srirama G, Konga YW, *et al.*** MK2 contributes to tumor progression by promoting M2 macrophage polarization and tumor angiogenesis. Proc Natl Acad Sci USA 2018; **115**: E4236-E44.

68. **Lyamina SV, Shimshelashvili ShL, Kalish SV, *et al.*** Changes in phenotype and phenotypic flexibility of alveolar macrophages in inflammatory pulmonary diseases. Pul'monologiya 2012; (6): 83–9 (in Russian).

69. **Kovaleva OV, Efremov GD, Mikhaylenko DS, *et al.*** Role of tumor-associated macrophages in renal cell carcinoma pathogenesis. Cancer Urology 2017; **13**: 20–6 (in Russian).

70. **Genard G, Lucas S, Michiels C.** Reprogramming of tumor-associated macrophages with anticancer therapies: radiotherapy versus chemo- and immunotherapies. Front Immunol 2017; **8**: 828.

71. **Komohara Y, Hasita H, Ohnishi K, *et al.*** Macrophage infiltration and its prognostic relevance in clear cell renal cell carcinoma. Cancer Sci 2011; **102**: 1424–31.

72. **Shu QH, Ge YS, Ma HX, *et al.*** Prognostic value of polarized macrophages in patients with hepatocellular carcinoma after curative resection. J Cell Mol Med 2016; **20**: 1024–35.

73. **Dong P, Ma L, Liu L, *et al.*** $CD86^+/CD206^+$, diametrically polarized tumor-associated macrophages, predict hepatocellular carcinoma patient prognosis. Int J Mol Sci 2016; **17**: 320.

74. **Jackute J, Zemaitis M, Pranys D, *et al.*** Distribution of M1 and M2 macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer. BMC Immunol 2018; **19**: 3.

75. **Xiaojuan W, Siyue N, Hui L, *et al.*** Angiopoietin-like protein 2 facilitates non-small cell lung cancer progression by promoting the polarization of M2 tumor-associated macrophages. Am J Cancer Res 2017; **7**: 2220–33.

76. **Rath M, Müller I, Kropf P, *et al.*** Metabolism via arginase or nitric oxide synthase: two competing arginine pathways in macrophages. Front Immunol 2014; **5**: 532.

77. **Ivanchenko AV, Symchych TV, Fedosova NI, *et al.*** Features of polarization of macrophages from different organs and tissues of mice with Lewis lung carcinoma. Oncology (Ukr) 2019; **21**: 63–4 (in Ukrainian).