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THE IMMUNE SYSTEM CONTRIBUTES TO THE EFFECTIVENESS OF VACCINE THERAPY IN PATIENTS WITH METASTATIC MELANOMA

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Abstract

The aim of the study was to identify differences in the immune system parameters between metastatic melanoma patients who responded and did not respond to dendritic cell vaccination. **Material and Methods.** The study group included 20 patients with stage III–IV metastatic melanoma, who received vaccine therapy with dendritic cells (DC) in a prophylactic mode. The control groups included 13 patients who had symptoms of disease progression at the time of starting vaccine therapy, and 5 healthy donors. The DC-vaccine was prepared in the form of a suspension of the patient’s autologous dendritic cells loaded with tumor antigens *in vitro*. A single dose had 2 million dendritic cells in 1 ml of phosphate buffer solution, which was administered intradermally in the nearest site to the regional lymphatic collectors. The immune system status was assessed before starting vaccination. The immune system status was evaluated according to the indices of 25 peripheral blood cell populations using multicolor flow cytometry and integral characteristic in the form of the visual image generated by the visualization method of multidimensional data (NovoSpark, Canada). **Results.** The immune status in patients with metastatic melanoma at the start of DC-vaccination differed and was associated with the effectiveness of subsequent vaccine therapy. The response to vaccination was observed in patients whose immune system status was similar to that of healthy individuals. Low efficacy of DC-vaccine therapy was shown in patients whose immune system status corresponded to that of patients with disease progression. Alterations of the immune system in patients with metastatic melanoma were registered both at the level of individual immunological parameters and at the level of visualized integral characteristics. The integral characteristics of the immune system associated with the patient’s immunocompromised status can be considered as a criterion for stratification of patients with metastatic melanoma for the effective DC-vaccine therapy. **Conclusion.** The effectiveness of vaccine therapy with dendritic cells in patients with metastatic melanoma is associated with the immune system state before starting this therapy.

Key words: immune system, melanoma, vaccine therapy, dendritic cells, method for visualizing multidimensional data.

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ИММУННАЯ СИСТЕМА ВНОСИТ ВКЛАД В ЭФФЕКТИВНОСТЬ ВАКЦИНОТЕРАПИИ У БОЛЬНЫХ МЕТАСТАТИЧЕСКОЙ МЕЛАНОМОЙ

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Аннотация

Целью исследования явилось выявление различия состояния иммунной системы у больных метастатической меланомой с ответом на лечение или без него. **Материал и методы.** Основную группу исследования составили 20 больных метастатической меланомой III–IV стадии, получавших вакцинотерапию дендритными клетками в профилактическом режиме. Группами контроля стали 13 пациентов, имевших признаки прогрессирования на момент назначения вакцинотерапии, и 5 здоровых доноров. Применяемая вакцина представляла собой суспензию аутологичных дендритных клеток пациента, нагруженных опухолевыми антигенами *in vitro.* Разовая доза препарата составляла 2 млн дендритных клеток в 1 мл фосфатного буферного раствора, которая вводилась внутрикожно в непосредственной близости от регионарных лимфатических коллекторов. Состояние иммунной системы исследовали до начала вакцинотерапии. О состоянии иммунной системы судили по популяционной структуре клеток периферической крови, полученной методом многоцветной проточной цитометрии, и по визуальному образу, представляющему интегральную характеристику состояния исследуемой системы и полученному с использованием метода визуализации многомерных данных NovoSpark (Канада). **Результаты.** Состояние иммунной системы у больных метастатической меланомой на этапе назначения вакцинотерапии дендритными клетками различалось и было связано с эффективностью последующей вакцинотерапии. Пациенты, ответившие на вакцинотерапию, характеризовались показателями, близкими к таковым у здоровых лиц. Низкая эффективность терапии дендритными клетками характерна для больных, состояние иммунной системы которых совпадало с таковым у больных с прогрессирующим процессом заболевания. Различия в иммунной системе у больных метастатической меланомой проявлялись как на уровне отдельных иммунологических параметров, так и на уровне интегральной характеристики, отражаемой визуальным образом. Интегральная характеристика состояния иммунной системы, отражающая степень ее компрометированности, может служить критерием стратификации больных с метастатической меланомой для назначения эффективной вакцинотерапии на основе дендритных клеток. **Заключение.** Эффективность вакцинотерапии дендритными клетками у больных метастатической меланомой связана с состоянием иммунной системы до начала данного вида лечения.

Ключевые слова: иммунная система, меланома, вакцинотерапия, дендритные клетки, метод визуализации многомерных объектов.

Introduction

Melanoma is a type of skin cancer of neuroectodermal origin that develops from the pigment-producing cells known as melanocytes. Melanoma is considered to be an immunogenic tumor capable of eliciting an effective immune response against antigens associated with the tumor. The feasibility of inducing an immune response is associated with a relatively frequent spontaneous regression of the primary and metastatic disease [1].

The involvement of immunological mechanisms in the pathogenesis of melanoma suggests the feasibility of using various immunotherapeutic approaches. One of the immunotherapeutic strategies used to treat melanoma is the use of dendritic cell-based vaccines. The ability of dendritic cells to induce specific CD4+ and CD8+ T cells in cancer patients is confirmed by numerous in vivo experiments, as well as clinical trials [2, 3]. Several vaccine trials have demonstrated clinical efficacy in patients with advanced melanoma, but overall efficacy remains low [1]. An increase in the effectiveness of immunotherapy can be facilitated by the identification of patient stratification criteria that predict the expected result of the response to this type of treatment.

R. Dronka et al. [4] suggested that the state of the immune system is a significant factor determining the effectiveness of immunotherapy in patients with melanoma. Based on clinical and experimental data that included 52 indicators of systemic immunity (29 cytokines and 23 cell populations), the authors showed that the complex and dynamic interaction between the tumor and the immune system of melanoma patients consists of alternating the phase of tumor rejection by the immune system and inhibition of the immune response by the tumor. The authors believe that vaccine therapy will be effective when administered in the phase when the immune system realizes its antitumor potential [4–6].

We have previously shown that the immune system contributes to the clinical course of malignant neoplasms and the effectiveness of antitumor treatment [7]. We substantiated an original approach for assessing the integral state of the immune system in the form of a visual image obtained on the basis of visualization of multidimensional data characterizing different parts of immunity (NovoSpark), and showed the significance of the integral characteristic for predicting the clinical course of malignant tumors [8].

The aim of the study was to identify the differences in the immune system status between melanoma patients who responded to dendritic cell-based vaccine therapy and patients who did not respond to this therapy

Material and Methods

The study included 33 patients with stage III–IV metastatic melanoma, who received standard systemic drug treatment (chemotherapy/chemoimmunotherapy). The study group consisted of 20 patients who had no evidence of disease progression. They received vaccine therapy with autologous dendritic cells in a prophylactic mode. Patients in this group had a satisfactory general condition (0–2 according to the ECOG/WHO scale); adequate blood counts and biochemical parameters reflecting the normal functioning of organs and systems; had no need for systemic glucocorticosteroids, had no other malignant neoplasms. Clinical and morphological parameters of patients of the study group are presented in Table 1.

The control group consisted of 13 patients who had signs of progression at the time of starting vaccine therapy. They received vaccine therapy with dendritic cells in a therapeutic regimen. Clinical and morphological parameters of the control group are presented in Table 2. The study also included 5 healthy

Table 1/Таблица 1

Characteristics of patients in the prophylactic group (AJCC 2002)

Характеристика пациентов профилактической группы (AJCC 2002)

Characteristics/Характеристики			Number/Количество
Number of patients (estimated)/Количество пациентов (оценено)			20
Gender/Пол			
Male/Муж			11
Female/Жен			9
Age(years)/Возраст, годы			28–76
Tumor location/Локализация первичной опухоли			
Trunk/Туловище			7
Extremities/Конечности			10
Head and neck/Голова и шея			2
Without identified primary tumor/Без выявленного первичного очага			1
Stage of the disease at the time of vaccination/ Стадия заболевания на момент вакцинотерапии	III	B	4
		C	7
		III_{Tx}	2
	IV	M1a	6
		M1b	1
		M1c	0

Table 2/Таблица 2

Characteristics of patients in the therapeutic group (AJCC 2002)
Характеристика пациентов терапевтической группы (AJCC 2002)

Characteristics/Признак			Number/Количество (n=13)
Gender/Пол			
Male/Муж			6
Female/Жен			7
Age, years/Возраст, годы			32–59
Disease stage/ Стадия заболевания	III	B	0
		C	0
	IV	M1a	2
		M1b	4
		M1c	7
Prior therapy for metastatic melanoma/ Предшествующая терапия по поводу метастатической меланомы	CT		13
	IT		9
	>1 line of drug therapy/>1 линии лекарственной терапии		13
	Did not receive drug treatment/Не получали лекарственного лечения		0
	More than 1 surgery/Более 1 операции		13

Note: CT − chemotherapy; IT – immunotherapy.

Примечание: ХТ − химиотерапия; ИТ – иммунотерапия.

Table 3/Таблица 3

The studied immunological parameters
Изучаемые иммунологические показателели

Population/Популяция	Population characteristics/Характеристика популяции
CD3+cells	Mature T-lymphocytes/Зрелые Т-лимфоциты
CD4+cells	Mature T-helpers/Зрелые Т-хелперы
CD8+cells	Mature cytotoxic T-lymphocytes/Зрелые цитотоксические Т-лимфоциты
CD4+/CD8+ratio	Immunoregulatory index/Иммунорегуляторный индекс
CD4+CD8+ cells	Minor subpopulation of activated T-lymphocytes, with a double positive phenotype/ Минорная субпопуляция активированных Т-лимфоцитов, с двойным позитивным фенотипом
CD3+CD4+ cells	T-helpers/Т-хелперы
CD3+CD8+ cells	Cytotoxic T-lymphocytes/Цитотоксические Т-лимфоциты
CD3-CD8+ cells	Subpopulation of natural killers/Субпопуляция натуральных киллеров
CD8+Perforin+ cells	Subpopulation of effector T-lymphocytes containing perforin lytic granules in their cytoplasm/ Субпопуляция эффекторных Т-лимфоцитов, содержащих в своей цитоплазме литические гранулы перфорина
CD8 active cells	The proportion of functionally active effectors in the structure of CD8+ cells/ Доля функционально активных эффекторов в структуре CD8+ клеток
CD16+ cells	Total population of natural killer cells/Общая популяция натуральных киллерных клеток
CD16+Perforin+ cells	Subpopulation of natural killer cells containing perforin lytic granules in their cytoplasm/ Субпопуляция натуральных киллерных клеток, содержащих в своей цитоплазме литические гранулы перфорина
CD16 active cells	The proportion of functionally active effectors in the structure of natural killer cells/Доля функционально активных эффекторов в структуре натуральных киллерных клеток
CD8+CD16+ cells	Natural killer cells, natural killer T-lymphocytes/ Натуральные киллерные клетки, натуральные киллерные Т-лимфоциты
CD3+CD16+CD56+ cells	Natural killer T lymphocytes/Натуральные киллерные Т-лимфоциты
CD3-CD16+CD56+ cells	Natural killer cells/Натуральные киллерные клетки
CD3-CD19+ cells	B-lymphocytes/B-лимфоциты
CD25+ cells	Activated cells/Активированные клетки
CD4+CD25+ cells	Activated helper T lymphocytes/Активированные хелперные Т-лимфоциты
HLA-DR+ cells	Activated cells/Активированные клетки
CD3+DR+ cells	Activated T-lymphocytes/Активированные Т-лимфоциты
CD28+ cells	Lymphocytes capable of interacting with antigen-presenting cells/ Лимфоциты, способные к взаимодействию с антиген-презентирующими клетками
CD8+CD28+ cells	Naive T-lymphocytes/Наивные Т-лимфоциты
CD11b+ cells	Effector lymphocytes/Эффекторные лимфоциты
CD8+CD11b+ cells	Effector T-lymphocytes/Эффекторные Т-лимфоциты
Perforin+ cells	Cells containing perforin lytic granules in their cytoplasm/ Клетки, содержащие в своей цитоплазме литические гранулы перфорина

donors. All patients signed a written informed consent before participating in the study.

Vaccine therapy was carried out as part of a phase I clinical trial with an assessment of the safety and efficacy of a dendritic vaccine within the framework of the Protocols approved by the Ministry of Health and Social Development of the Russian Federation, in the Department of Tumor Biotherapy of N.N. Blokhin Russian Cancer Research Center in compliance with GCP (Good clinical practice) standards (permission dated 08/06/2008 No. 371 and 06/21/2010 No. 286 of the Ministry of Health and Social Development of the Russian Federation).

The vaccine used is a suspension of the patient's autologous dendritic cells loaded with tumor antigens in vitro and reintroduced back to the patient. The vaccine for each patient participating in the study was prepared individually according to a general formalized protocol [9]. A single dose of the drug was 2 million dendritic cells in 1 ml of phosphate buffer solution, which was administered intradermally in close proximity to regional lymphatic collectors (shoulders, scapular region, thighs and abdomen).

Assessment of the status of the immune system

The immunological study was a multicolor phenotyping of peripheral blood cells by flow cytometry using monoclonal antibodies to CD3, CD4, CD8, CD16, CD56, CD19, CD25, CD28, CD11b, HLA-DR, and Perforin molecules. The studied molecular markers made it possible to identify 25 populations among circulating peripheral blood cells that differed in phenotypic and functional characteristics (parameters are presented in Table 3). The immunoregulatory index was calculated as CD4+/CD8+ ratio. Quantitative cytometric analysis was performed using a FACSCalibur flow cytometer (BDBiosciences, USA). At least 5000 events in the gate of CD45+/CD14- lymphocytes were accumulated and analyzed in each sample. The number of cells with the CD45+/CD14- phenotype was at least 95–97 %. Dot plot analysis was used, the relative number of positive cells (%) was taken into account. Appropriate isotype controls were used to discriminate against non-specific binding. Immunological parameters were assessed prior to treatment.

Method for visualizing multidimensional data

In our study, the status of the immune system, characterized by the phenotypic features of peripheral blood cells (n=26), was considered in each individual as a multidimensional dimension. For this, NovoSpark Corporation (Canada), the multidimensional data visualization method, was used. Visualization of a biosystem status, as a whole, involves displaying the totality of all parameters describing the state of the system in one "integral" image [10]. This method of visualization of multidimensional objects and processes is based on the isometry of two spaces, where objects of one space (data space) are considered originals, while another space objects play the role of images. The basis for identifying similarities or differences in the original data is the visual proximity of the images corresponding to these data, expressed in the Mahalanobis distance [8].

Fig. 1 shows an example of the presentation of three different cases, each of which is characterized by a certain set of immunological parameters (table at the bottom of the figure) and their corresponding visual image. Due to differences in immunological parameters, visual images have a different shape and location in multidimensional space. By entering 26 studied immunological parameters of each patient into the table of the NovoSpark Visualizer software,

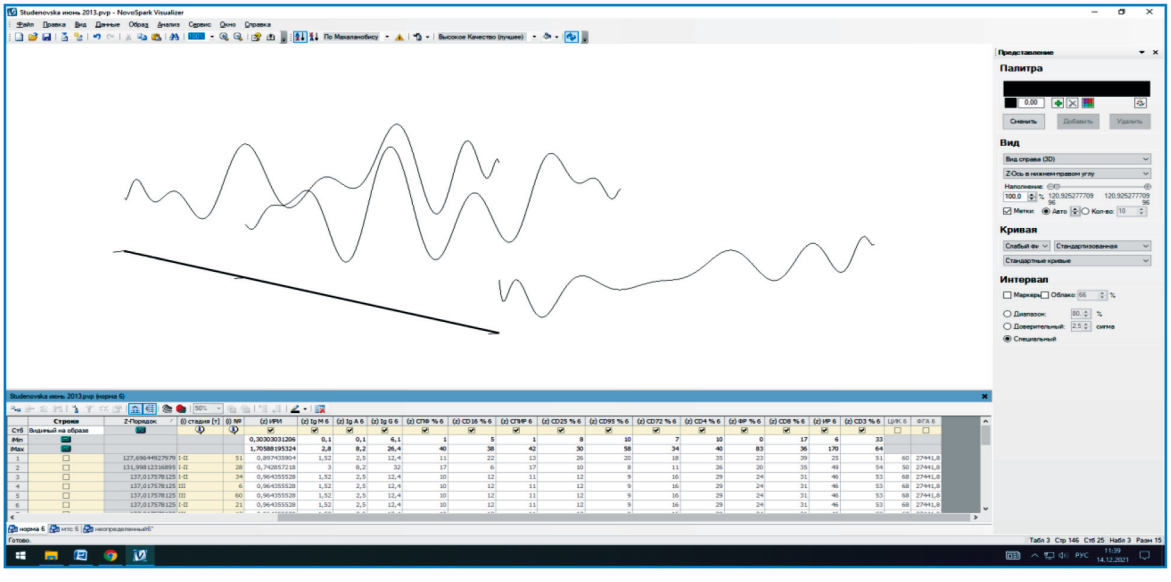

Fig. 1. An example of presenting the immune system status as a visual image using the NovoSpark Vizualizer software
Рис. 1. Пример представления состояния иммунной системы в виде визуального образа при использовании программы NovoSpark Vizualizer

we obtained individual curves having their own shape and position in the multidimensional data space, which are characteristics of the state of the immune system of each patient.

Statistical analysis was carried out using the standard Statistica 10 software package. To assess the significance of sample differences, the nonparametric Mann-Whitney test was used. P-values below 0.05 were considered statistically significant, and P-values below 0.1 were used as a statistical trend. To classify and describe the status of the immune system in patients with metastatic melanoma with different responses to vaccine therapy, the method of discriminant analysis was used. The statistical significance of the discriminatory power of the discriminant function was assessed using the value of Wilks' lambda [11].

Results

Survival rates in patients with melanoma who received prophylactic vaccine therapy

Vaccine therapy was carried out between 2005 and 2009. The follow-up time exceeded 156 months from the beginning of the study. The group of metastatic melanoma patients who received prophylactic vaccine therapy was heterogeneous in terms of the overall and disease-free survival rates. Of 20 melanoma patients, 13 showed no signs of disease progression during the entire follow-up period, all of them were alive at the time of the last examination. The relapse-free survival rates in the remaining 7 patients were significantly lower: median relapse-free and overall survival rates were 18 and 33 months, respectively (Fig. 2, 3, $p<0.001$ and $p=0.006$ for relapse-free and overall survival, respectively). The identified differences in survival were the basis for characterizing patients with long-term survival as responders to vaccine therapy, and patients with a short period of overall and disease-free survival as non-responders to this therapy.

State of the immune system in melanoma patients with different response to dendritic cell vaccine

The study revealed significant differences in the immunological parameters in patients who received prophylactic dendritic cell-based vaccine therapy (Table 4). Patients who responded to vaccine therapy had higher levels of CD3+, CD4+, CD3+4+, CD4+25+ lymphocytes, as well as higher levels of CD4/CD8 immunoregulatory index ($p<0.05$). However, populations of specialized cytotoxic lymphocytes, such as CD8+, CD3-8+, and PF+ (perforin secreting cells) were numerically smaller in responders than in non-responders at the level of statistical trend ($p<0.1$).

Visualization of the state of the immune system in metastatic melanoma patients with different responses to vaccine therapy

Visualization of the immune system state as a multidimensional observation makes it possible to demonstrate a qualitative difference in the immunological status in cancer patients [7, 12–14]. It is important that this methodological approach allows identification of immunological parameters that are statistically significant for discrimination of the immune system state in groups with various clinical manifestations.

Initially, when imaging the immune system in patients with metastatic melanoma, we used the entire range of studied immunological parameters. As can be seen in Fig. 4, visual images characterizing the immune system state in patients with and without response to vaccine therapy are located in overlaping regions of the space of multidimensional features.

Using the method of removing variables [12], we obtained the location of images of the immune system state of patients from the compared groups in different (non-overlapping) regions of the space of multidimensional features (Fig. 5). Therefore, despite the presence of common mechanisms for the involvement of the immune system in response to the presence of a tumor, there are marked differences in the immune system state in patients with or without response to dendritic cell-based vaccine therapy.

Discriminant model of the immune system status in patients with metastatic melanoma with different efficacy of dendritic cell vaccine therapy

For a formal description of the identified types of the immune system state, immunological parameters, the inclusion of which in the model provided a visual separation of the images of the immune system in patients with different responses to vaccine therapy, were used

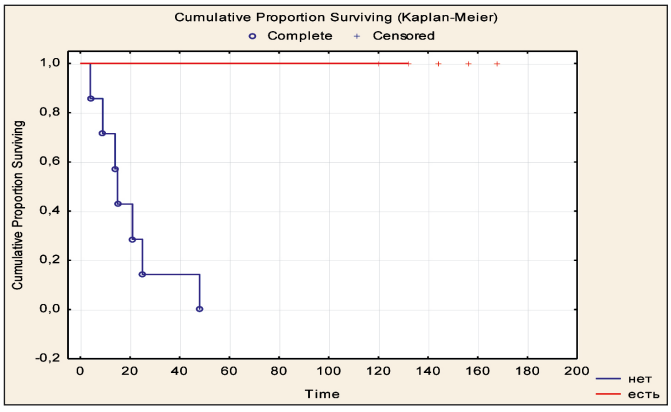

Fig. 2. Relapse-free survival in patients with metastatic melanoma who received prophylactic vaccine therapy in relation to the effectiveness of treatment.
Note: blue color – patients who did not respond to vaccine therapy, red color – patients who responded to vaccine therapy
Рис. 2. Безрецидивная выживаемость у больных метастатической меланомой, получавших вакцинотерапию в профилактическом режиме, в зависимости от эффективности лечения.
Примечание: голубой цвет – пациенты, не ответившие на вакцинотерапию, красный цвет – пациенты, ответившие на вакцинотерапию

Table 4/Таблица 4

Immunological parameters in patients with melanoma associated with the response to vaccine therapy, Me (LQu–UpQu)

Иммунологические показатели у больных меланомой в зависимости от ответа на вакцинотерапию, Me (LQu–UpQu)

Immunological parameters/ Показатели	Patients who responded to vaccine therapy/ Пациенты, ответившие на вакцинотерапию	Patients who did not respond to vaccine therapy/ Пациенты без ответа на вакцинотерапию
CD3, %	**73.30 (68.90–77.40)**	**59.95 (54.80–67.90)****
CD4, %	**42.20 (35.40–45.90)**	**34.80 (28.20–37.50)****
CD8, %	**32.90 (28.90–36.80)**	**38.60 (33.30–45.50)***
CD4/CD8	**1.30 (1.10–1.40)**	**0.80 (0.70–0.90)***
CD16, %	27.00 (22.80–30.20)	31.20 (18.90–36.60)
CD3-19+, %	6.90 (5.40–9.70)	9.20 (5.90–15.60)
CD3+4+, %	**42.20 (35.40–45.50)**	**34.55 (28.20–36.40)****
CD3+8+, %	25.60 (24.00 – 29.00)	25.65 (17.80–30.10)
CD3-8+, %	**6.60 (5.00–7.50)**	**12.75 (5.20–18.00)***
CD4+8+, %	3.20 (1.70–3.70)	4.90 (1.90–6.30)
CD8+16+, %	8.60 (7.00–9.70)	10.90 (4.40–20.10)
CD3+16+56+, %	6.40 (4.20–8.90	5.15 (2.60–12.30)
CD3-16+56+, %	20.60 (16.30–22.20)	21.25 (16.70–26.60)
CD25+, %	19.20 (14.40–26.30)	13.70 (9.20–22.10)
CD4+25+, %	**9.40 (9.00–13.90)**	**6.95 (5.10–8.10)****
HLA-DR+, %	16.00 (10.20–17.10)	18.75 (10.30–36.90)
CD3+DR+, %	7.20 (6.20–9.40)	7.30 (3.10–18.10)
CD28+, %	41.10 (35.10–48.80)	43.60 (42.30–57.70)
CD8+CD28+, %	17.50 (14.30 ± 20.50)	16.95 (10.80–30.00)
CD11b+, %	51.30 (31.00–62.00)	35.75 (30.20–49.60)
CD8+11b+, %	12.10 (7.4–17.50)	8.95 (6.40–21.40)
PF+, %	**27.60 (24.70–32.00)**	**36.45 (30.00–42.50)***
CD8+PF+, %	12.30 (10.70–15.10)	13.35 (9.2–20.50)
CD16+PF+, %	18.00 (15.700–23.30)	24.05 (18.30–30.60)
CD8active, %	40.30 (39.10–44.20)	39.65 (23.10–47.60)
CD16active, %	65.80 (60.20–78.90)	78.45 (55.60–93.10)

Note: * – statistical trends (p<0.1); ** – statistical differences (p<0.05).

Примечание: * – статистические различия p<0,1; ** – различия статистически значимы (p<0,05).

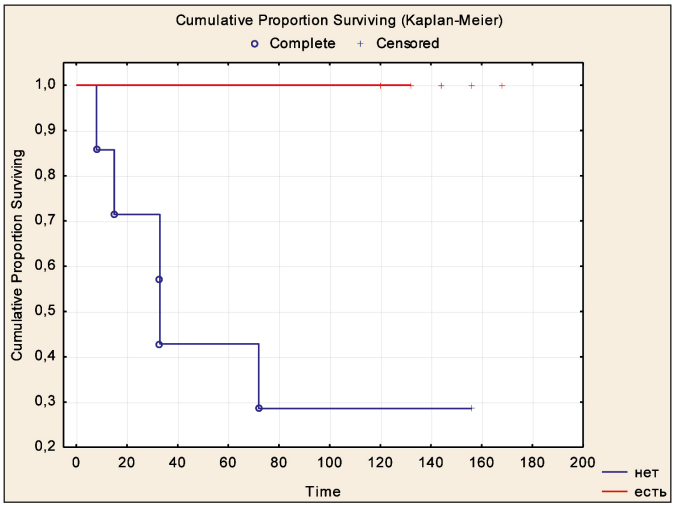

Fig. 3. Overall survival in patients with metastatic melanoma who received prophylactic vaccine therapy in relation to the effectiveness of treatment. Note: blue color – patients who did not respond to vaccine therapy, red color – patients who responded to vaccine therapy

Рис. 3. Общая выживаемость у больных метастатической меланомой, получавших вакцинотерапию в профилактическом режиме, в зависимости от эффективности лечения. Примечание: голубой цвет – пациенты, не ответившие на вакцинотерапию, красный цвет – пациенты, ответившие на вакцинотерапию

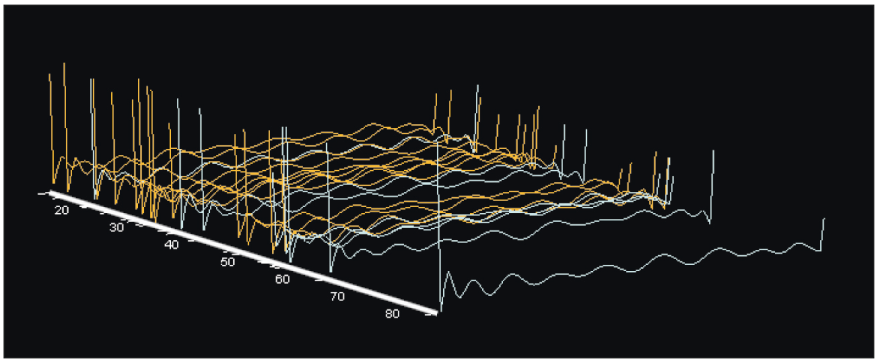

Fig. 4. Location of visual images representing the immune system state in patients with advanced melanoma without selection of significant variables. Note: Immune system images are shown in orange in melanoma patients who responded to vaccine therapy, in blue – in patients who did not respond to vaccine therapy

Рис. 4. Расположение визуальных образов, отражающих состояние иммунной системы у больных метастатической меланомой без отбора значимых переменных. Примечание: оранжевым цветом представлены образы иммунной системы у больных меланомой, ответивших на вакцинотерапию, голубым цветом – у пациентов без ответа на вакцинотерапию

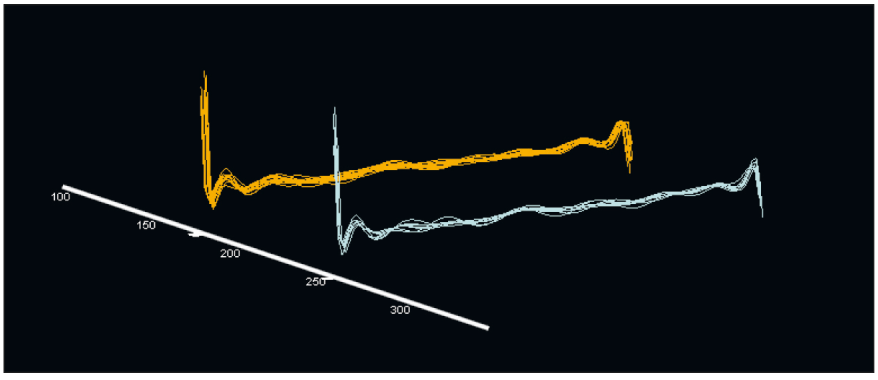

Fig. 5. The location of visual images reprsenting the immune system state in patients with advanced melanoma after the selection of significant variables. Note: Immune system images are shown in orange in melanoma patients who responded to vaccine therapy, in blue – in patients who did not respond to vaccine therapy

Рис. 5. Расположение визуальных образов, отражающих состояние иммунной системы у больных метастатической меланомой после отбора значимых переменных. Примечание: оранжевым цветом представлены образы иммунной системы у больных меланомой, ответивших на вакцинотерапию, голубым цветом – у пациентов без ответа на вакцинотерапию

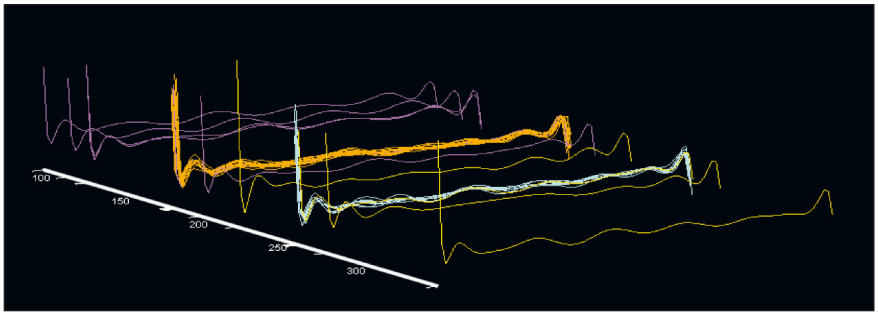

Fig. 6. Visualization of the immune system status in patients who responded (orange) and did not respond (blue) to vaccine therapy in relation to visual images of the immune system in healthy individuals (violet) and in patients with advanced melanoma (green)

Рис. 6. Визуализация состояния иммунной системы у пациентов, ответивших (оранжевый цвет) и не ответивших (голубой цвет) на вакцинотерапию, относительно визуальных образов иммунной системы у здоровых лиц (фиолетовый цвет) и у больных меланомой в состоянии прогрессирования (зеленый цвет)

as variables for the classification discriminant functions. The type of classification functions is shown in Table 5. Wilks' lambda was 0,022 (χ^2=43,79, p=0,001).

Variables excluded from the parameters when achieving visual separation of the immune system images in patients, depending on the response to vaccine therapy during the discrimination procedure, were the following immunological parameters: CD3+CD4+, CD3+CD8+, CD3-CD8+, CD8+Perforin+, CD16+Perforin+ , CD8activ, CD16activ peripheral blood cells.

Table 5/Таблица 5

Immunological parameters and their corresponding coefficients included in the classification discriminant functions of the immune system status in patients with advanced melanoma in relation to the effectiveness of vaccine therapy

Иммунологические показатели и соответствующие им коэффициенты, входящие в классификационные дискриминантные функции состояния иммунной системы у больных с распространенной меланомой в зависимости от эффективности вакцинотерапии

	Response to vaccine therapy/ Ответ на вакцинотерапию	No response to vaccine therapy/ Отсутствие ответа на вакцинотерапию
Parameters/Параметры	-808.168	-1051.58
CD3	11.867	14.11
CD4	4.015	3.51
CD8	18.682	28.45
ИРИ	95.549	114.66
CD16	8.093	-8.27
CD3-CD19+	-50.199	-62.10
CD4+CD8+	-8.355	-9.18
CD8+CD16+	-0.963	7.01
CD3+CD16+CD56+	-20.207	-13.96
CD3-CD16+CD56+	-2.223	11.49
CD25+	1.232	4.71
CD4+CD25+	-10.346	-22.19
HLA-DR+	57.587	65.53
CD3+DR+	-48.709	-49.62
CD28+	-6.815	-10.79
CD8+CD28+	9.543	11.17
CD11b+	-4.071	-6.62
CD8+CD11b+	1.759	-2.37
Perforin+	8.205	12.48

Comparison of the immune system state in melanoma patients with the health status of individuals and in patients with disease progression

We conducted a comparative analysis of the immune system state in melanoma patients with different responses to vaccine therapy with the immune system state in healthy individuals with no history of malignant neoplasms, and in patients with metastatic melanoma who had signs of disease progression at the time of vaccination appointment and receiving vaccine therapy.

Imaging of the immune system state showed that visual images in patients who responded to vaccine therapy were located in the area of the immune system state in healthy individuals, while in patients who did not respond to treatment, the location of the images coincided with the area in melanoma patients who had disease progression before starting vaccine therapy (Fig. 6).

The classification discriminant function characterizing the immune system status in patients who responded to vaccine therapy was applicable in 100 % of cases to describing the status of healthy individuals, and the function describing the health status in patients who did not respond to vaccine therapy classified patients with manifested metastasis in 80 % of cases.

Discussion

Melanoma is an immunogenic tumor, for which an immune response to tumor-specific antigens has been found [15]. A number of studies have shown the correlation of various parameters characterizing the immune response, both with the parameters of tumor progression and with overall and relapse-free survival in patients with melanoma [16, 17], on the basis of which a number of authors make a conclusion about the significance of immunological mechanisms in the pathogenesis of this disease [16–19].

It is quite obvious that the effectiveness of melanoma immunotherapy is closely related to the functional state of the immune system. Thus, in a study by T. García-Salum et al. (2018), which included 28 patients with advanced melanoma who received vaccine therapy with dendritic cells loaded with antigens from lysed melanoma cells, significant differences in the immunological parameters between patients who responded or did not respond to treatment were found [20]. It is assumed that in malignant neoplasms, effective immunotherapy and, in particular, dendritic cell-based vaccine therapy, is hindered by compromising the functional status of the immune system by the present tumor [21].

S.M. Lluesma et al. (2018) showed that if the immune system was competent against tumors, including melanoma, then this status positively

affected the therapeutic efficacy of dendritic cell-based vaccines [3]. Therefore, the compromise of the immune system under the influence of a tumor can be a significant obstacle to successful immunotherapy [3]. The compromise may be associated with the induction of immunosuppression by the tumor [21], inhibition of cytotoxic mechanisms against tumor cells, blocking the recognition of tumor antigens, and other effects associated with the escape of the tumor from an effective immune response [3, 22–26].

The study revealed statistically significant phenotypic differences in peripheral blood cells in patients with melanoma, depending on the effectiveness of vaccine therapy with dendritic cells. In patients with a response to vaccine therapy, the number of CD3+ T-lymphocytes as the main effectors of cellular cytotoxicity before treatment was higher than in patients without a response to therapy (72.61 ± 2.14% and 61.04 ± 2.62%, respectively, $p<0.050$). Paradoxically, the level of cytotoxic T-lymphocytes (CD8+ lymphocytes) and cells producing perforin, an effector molecule of cellular cytotoxicity (PF+ cells), was lower in melanoma patients who responded to vaccine therapy than in non-responders at the level of statistical trend. The revealed phenomenon suggests the induction of antitumor cytotoxic clones of lymphocytes in the process of vaccine therapy. The activation or the presence of these populations before treatment are unfavorable signs indicating the potential induction of T-regulatory cells during dendritic cell vaccination, which, in turn, leads to blocking of the Th1 immune response [27].

Despite the fact that the significance of antitumor defense mechanisms distorted by the presence of a tumor is actively discussed in the literature, there are very few reports regarding the exact parameters reflecting the fact that the immune system is compromised. R. Dronca et al. have shown that the immune system responds to the presence of metastatic melanoma systemically, which is reflected in the synchronized changes in a large number of immunological parameters, including the level of cytokines, chemokines, and lymphocyte subpopulations in peripheral blood [4]. This is consistent with our results when we used the method of visualizing the immune system state as an integrated unite, allowing reflection of systemic relationships that determine a single strategy for the response of the immune system. This approach showed differences in the immune system state in cancer patients with different risks of progression and substantiated the feasibility of using the integral characteristic of the immune system as a prognostic criterion [7, 14, 28]. The method of visualization of the immune system state in patients with melanoma showed differences depending on the response to vaccine therapy (Fig. 5). Among the immunological parameters excluded from the classification discriminant equations obtained from visualization, there were parameters, such as the number of cytotoxic lymphocytes (CD8+ cells) and perforin-producing lymphocytes (PF+ cells). In other words, these populations are not important for discrimination of the immune system state as a significant factor for effective vaccine therapy, but are probably associated with the presence of a tumor.

The response to vaccination was observed in patients whose immune system state was similar to that of healthy individuals. Visual images of the immune system in patients with advanced melanoma matched those in patients who did not respond to vaccine therapy (Fig. 6). Since there was no compromise of the immune system in relation to the tumor in healthy individuals, while in patients with tumor progression it was expressed to the maximum extent, the results obtained confirmed the conclusion made by S.M. Lluesma et al. (2018) in [3], that the lack of response to DC-vaccine therapy was explained by disorders of the immune system. The classification discriminant functions obtained in our study act as those missing characteristics of immunocompetence or immunocompromise in patients with melanoma, which are mentioned by T. Whiteside [21]. Their use will allow assessment of the immune state, on the basis of which dendritic cell vaccine therapy is indicated or not indicated in patients with melanoma.

Conclusion

The immune system state in patients with metastatic melanoma at the stage of dendritic cell-based vaccine therapy appointment is represented by different types. It was revealed that the type of the immune system state is associated with the effectiveness of subsequent vaccine therapy. The response to vaccine therapy was observed in patients whose immune state is close to that of healthy individuals. The low efficacy of dendritic cell therapy is typical for patients whose immune system state is similar to that of patients with progressive disease. Differences in the immune system in patients with metastatic melanoma are manifested both at the level of individual immunological parameters and at the level of an integral characteristic reflected visually. An integral characteristic of the immune system state, reflecting the degree of its compromise, can be a criterion for stratifying patients with metastatic melanoma for administering effective dendritic cells-based vaccine therapy.

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Marina N. Stakheyeva: Project development, data collection and interpretation, critical revision of the manuscript for important intellectual content.
Irina Zh. Shubina: writing of the manuscript, data collection and interpretation.
Georgi Z. Chkadua: development of an antitumor vaccine; participation in clinical trials.
Anna A. Borunova: participation in clinical trials (study of the immunological status of patients).
Ruslan A. Zukov: research supervision, critical revision with the introduction of valuable intellectual content.
Igor V. Bogdashin: data analysis and interpretation
Evgeny L. Choynzonov: data analysis and interpretation, critical revision with the introduction of valuable intellectual content.
Nadezhda V. Cherdyntseva: data analysis and interpretation, critical revision with the introduction of valuable intellectual content.
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Conflict of interests
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ВКЛАД АВТОРОВ

Михайлова Ирина Николаевна: проведение клинических исследований**;** разработка концепции научной работы, поиск источников литературы, анализ и интерпретация научной работы, критический пересмотр с внесением ценного интеллектуального содержания.

Стахеева Марина Николаевна: разработка концепции научной работы, поиск источников литературы, анализ и интерпретация полученных данных, критический пересмотр с внесением ценного интеллектуального содержания.

Шубина Ирина Жановна: написание текста статьи; поиск источников литературы, анализ и интерпретация научной работы.

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Чойнзонов Евгений Лхамацыренович: анализ и интерпретация научной работы, критический пересмотр с внесением ценного интеллектуального содержания.

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