Journal of Stem Cells
Volume 10, Number 4

ISSN: 1556-8539

Regeneration and Carcinogenesis

A. V. Pechersky[1,*], V. I. Pechersky, A. B. Smolyaninov[2], V. N. Velyaninov[3], S. F. Adylov[2], A. Yu. Shmelev[1], O. V. Pecherskaya[1], and V. F. Semiglazov[4]

[1]North-West State Medical University named after I.I. Mechnicov, St. Petersburg, Russia
[2]Pokrovsk Bank of Stem Cells, St. Petersburg, Russia
[3]Army Medical Academy named after S.M. Kirov, St. Petersburg, Russia
[4]N.N. Petrov Scientific Research Institute of Oncology, St. Petersburg, Russia

Abstract

Introduction. Once people reach 40 years of age, they have a decrease in their pool of pluripotent stem cells, and an increased risk for development of oncological diseases.
Materials and methods. The first part of the study was conducted in 11 patients aging 54 to 76 years old with cancer of the kidney, bladder, or prostate in stages III-IV of the disease. The second part of the study was conducted in four patients aged 60-82 years old, who were given from 4 to 7 transfusions of mononuclear fraction of peripheral blood from young donors 19-23 years old, with the same sex and blood types as the recipients, in order to restore cell regeneration.
Results. In the first part of the study, 1 month after chemotherapy or targeted therapy, all 11 cancer patients had leukopenia accompanied by an increase in the contents of FGFb in the blood by 1.74 times on average. Four of these patients had an increase in the level of human VEGF-A of 1.25 times on average, while three patients had an increase in the level of human EGF of 1.13 times on average. In the second part of the study, 3-6 months after the completion of a cycle of 4-7 blood transfusions of mononuclear fraction of periphery blood, four patients had an in increase in the contents of hematopoietic progenitor cells $CD34^+$ of periphery blood by 3.25 times on average, to the level normal in young people, while the level of FGFb decreased by 1.78 times on average. Among two patients, the level of human VEGF-A decreased by 1.48 times on average, while for three patients the level of human EGF decreased by 4.12 times on average. In the buccal epithelium, all four patients had a decrease in the expression of p53 by 6.02 times on average, while three of them had a decrease in the expression of Bcl-2 by 60.0 times on average.
Conclusion. Violation of tissue renewal is a major cause of carcinogenesis in people older than 40 years old. Excessive stimulation of mitotic activity among people over 40 can be reduced to normal levels by restoring the pool of pluripotent stem cells through transfusion of mononuclear fraction of peripheral blood from young donors 18-23 years old with the same blood groups and sex as the recipient (RF patent number 2350340).

Keywords: regeneration, carcinogenesis, pluripotent stem cells, testosterone, AR, FGFb, Bcl-2, p53

* Corresponding Author: Dr. Alexander Pechersky, Email: a_pechersky@mail.ru

Introduction

Once people reach 40 years of age, they have a decrease in their pool of pluripotent stem cells [1], leading to atrophy and fibrosis in all tissues and organs, as well as an increased risk of cancer [2, 3, 4]. A study of the link between failed tissue renovation and carcinogenesis is of considerable interest.

Objective

Study the causes and ways to normalize increased mitogenic stimulation in people over 40 years old.

Materials and methods

Studying the dependency of mitogenic stimulation on changes in tissue renewal (regeneration) in people over 40 years old was carried out using the example of intensification of existing violations in regeneration among cancer patients receiving chemotherapy/targeted therapy, and using the opposite example of the recovery of regeneration in patients older than 50 years old who were provided with a transfusion of mononuclear fraction of peripheral blood from young donors 19-23 years old. In both the first and second cases, changes in mitogenic stimulation occurred over a relatively short period of time, which suggested they were connected to worsening regeneration disorders, or, alternatively, to restoration of regeneration.

In the first case, mitogenic factors – cell growth factors were determined in 11 patients aged 54 to 76 years old with cancer of the kidney, bladder, or prostate cancer in the III – IV stages of the disease before and 1 month after the initiation of chemotherapy or targeted therapy. Since pluripotent stem cells are involved in the renewal of all body tissues [4], when there is suppression of the pool of stem cells during chemotherapy/targeted therapy (as confirmed by the development of leukopenia), all patients showed a violation of tissue renewal (regeneration disorder). The criteria for inclusion in the main group were the following: undergoing chemotherapy or targeted therapy for various cancers, and the patients' age, which was over 50 years old. The control group consisted of 11 apparently healthy blood donors between the ages of 18 to 23 years old. Selection criteria were the following: apparently healthy people aged 18 to 25 years old.

In the second case, mitogenic factors – cell growth factors were detected in four patients aged 60 to 82 years old, who received from four to seven transfusions of mononuclear fraction of peripheral blood from young donors 19 to 23 years old at intervals of 2 to 3 months, with the aim of restoring the tissue renewal process (restoration of regeneration).

The criteria for inclusion in the main group were the following: patient age older than 50 years old and transfusion to these patients of allogeneic mononuclear fraction peripheral blood in order to restore regeneration. Procurement of mononuclear fraction peripheral blood for each of the four recipients was made from one young donor 19-23 years old. The donor selection criteria were the following: compliance to the health requirements of the Order of the Ministry of Health of the Russian Federation dated September 14, 2001 № 364 "On approval of the medical examination of donor blood and its components," an age of 18 - 23 years old, the same gender as the recipients, and the same antigen systems AB0, Rh-factor, phenotype Rh-factor, and Kell (Russian Federation patent 2350340) as the recipients. For Kell (+), transfusion was carried out from a donor with Kell (-). The control group consisted of four healthy blood donors between the ages of 19 to 22 years old. The selection criteria were the following: apparently healthy people aged 18 to 25 years old.

Evaluating the effectiveness of treatment aimed at restoring regeneration was carried out based on determining the number of hematopoietic progenitor cells $CD34^+$ of peripheral blood. Additionally, after obtaining patients' consent, sampling of biopsies of oral mucosa was performed under local anesthesia for conducting histological and immunohistochemical studies. The choice of the buccal epithelium was random, and was conditioned by the simplicity of sampling this material. Changes in the buccal epithelium were interpreted as a reflection of overall changes in the tissues of patients receiving transfusions of mononuclear fraction of peripheral blood.

Procurement of donors' mononuclear fraction of peripheral blood was carried out using an Amicus separator and an Amicus MNC-Kit with a specific one-use sterile expenditure system. During the first transfusion, in order to form a chimerism to minimize the antigen load, there was a reduced intake of mononuclear fraction of peripheral blood: 5 cycles of separation (the approximate volume of intraoperative treated blood of the donor–1,500 ml). For subsequent transfusions, carried out in order to restore tissue renewal (regeneration restoration), mononuclear fraction of peripheral blood was obtained by carrying out 15 separation cycles with processing of 4,600 ± 100 ml of donor blood. Settling of erythrocytes was performed using 20 ml of Stabizol hemocorrector solution. Donors underwent a standard checkup before procurement of mononuclear fraction of peripheral blood was conducted. Donors were prepared for five days before the leucopheresis operation. Donors, who were men with a body weight of 75 kg on average, were injected subcutaneously with filgrastim (brand name – Neipomax®) in a dose of 78 mln units/30 + 48 mln units (780 μg/300 + 480 μg) once per day, daily, over the course of 5 days. For women, who had a lower body weight than men (60 kg on average), the dose of filgrastim (Neipomax®) was 60 mln units/30 + 30 mln units (600 μg/300 + 300 μg) once per day, daily, over the course of 5 days. The last filgrastim administration was done on the day of separation. At the end of the transfusion of mononuclear fraction of peripheral blood, patients were administered one dose of 50 mg of Prednisolone intravenously to reduce the severity of post-transfusion reactions. Starting from the first hours after transfusion to patients – men with a body weight of 80 kg on average – filgrastim (Neipomax®) was subcutaneously injected at a dose of 78 mln units/30 + 48 mln units (780 μg/300 + 480 μg) once per day, daily, over the course of 10 days. For women, who had a lower body weight than men (60 kg on average), the dose of filgrastim (Neipomax®) was 60 mln units/30 + 30 mln units (600 μg/300 + 300 μg) once per day, daily, over the course of 10 days. Injecting colony-stimulating factor (filgrastim) was done for transfused mononuclear leukocytes to overcome the restriction point as they enter the alien blood environment of the recipient, as well as for subsequent stimulation of their division and an increase in their numbers. Additionally, for the same purpose, patients were administered Methyluracil tablets 0.5 g, 1 tablet, four times a day, for 1 month.

Research Methods

Measuring peripheral blood hematopoietic $CD34^+$ progenitor cells was performed by flow cytometry on a FC500 flow cytometer using a set of Stem-Kit Reagents made by Beckman Coulter Company (France). The sensitivity of the method for determining the concentration of peripheral blood hematopoietic $CD34^+$ progenitor cells was 0.5 cells/mcl. The coefficient of variation was 10.7%.

Enzyme immunoassay examination. Measuring human fibroblast growth factor with a basic shape (FGFb) in the blood serum was carried out using a test set made by the company R & D Systems, Inc., with a test-sensitivity of 3.0 pg/ml, and a coefficient of variation of 5.3%. Measuring human vascular endothelial growth factor A (VEGF-A) was carried out using a test set made by Bender MedSystems, with a test-sensitivity of 7.9 pg/ml, and a coefficient of variation of 6.2%. Measuring human epidermal growth factor (human EGF) was carried out using a test set made by Bender MedSystems, with a test-sensitivity of 0.26 pg/ml, and a coefficient of variation of 3.5%.

Morphological examination. Pieces of the oral mucosa were fixed in a 10% solution of neutral formalin, were put through alcohols, and were embedded in paraffin according to standard methods of histological preparations [5]. Sections 5 μm thick were stained with hematoxylin and eosin (H&E section).

Immunohistochemical studies. Measuring the expression of androgen receptors (AR), as well as the expression of p53 and Bcl-2 was carried out using a one-step method for unmasking the antigen (using a method of high-temperature tissue treatment) on paraffin sections using a diagnostic kit produced by Novocastra Laboratories Ltd (Great Britain) for AR and p53, and by Dako (Denmark) for Bcl-2. AR identification results were assessed using a semiquantitative histochemical score method [6]. The values of p53 and Bcl-2 are presented as a percentage of the number of positive cells to 1000 cells scanned.

Statistical analysis. Statistical analysis was performed using the methods of variance analysis of repeated measures and variance analysis for comparing two groups using Student's t-test. All data in the text and tables are presented as average values and standard deviations (M ± σ). Furthermore, Student's t-test (t) values are presented [7].

Results and Discussion

In the first part of the study, 1 month after chemotherapy or targeted therapy, all 11 cancer patients developed leukopenia, accompanied by an increase in basic fibroblast growth factor (FGFb) in the blood by an average of 1.74 times. That said the original average level of basic fibroblast growth factor (FGFb) before treatment in all 11 cancer patients was 5.5 times higher than the analogous level among young people in the control group (Tables 1, 2). Also, among cancer patients of the main group, following chemotherapy/targeted therapy there was a tendency towards an increase in the levels of human vascular endothelial growth factor A (VEGF-A) and human epidermal growth factor (human EGF). Thus, of the 11 cancer patients, 4 of them had an increase in the level of human vascular endothelial growth factor A (VEGF-A) by 1.25 times on average (from 351.1 ± 189.7 pg/ml to 439.4 ± 161,7 pg/ml), while 3 patients had an increase in the level of human epidermal growth factor (human EGF) by 1.13 times on average (from 217.8 ± 22,9 pg/ml to 246.4 ± 2,5 pg/ml) (Tables 3, 4).

In the second part of the study, 3 to 6 months after completing the cycle of 4 to 7 transfusions of mononuclear fraction of peripheral blood, 4 patients showed an increase in hematopoietic progenitor cells $CD34^+$ of peripheral blood by 3.3 times on average (from 1 to 2 - 5 cells in 1 mcl, to 3.3 cells per 1 mcl on average). A comparison of the result received with the instructions of Stem-Kit Reagents by Beckman Coulter (France) on 117 healthy individuals of various age groups with the maximum level of $CD34^+$ of peripheral blood among young people – 6.5 Cells/mcl, and the minimal level among older individuals – 0.5 Cells/mcl, showed that among patients who received the cycle of transfusions of mononuclear fraction of peripheral blood from donors 19 – 23 years old, the level of hematopoietic progenitor cells $CD34^+$ of peripheral blood increased to a level characteristic of young men. This indicates that it was possible among 60 - 82 year old patients to restore the stem cell pool to the level of young individuals (Table 5, 8). After restoration of the pool of stem cells among 4 patients from 60 - 82 years old, and therefore of the regeneration process, to the level among young people, 3 to 6 months after completing the cycle of transfusions of mononuclear fraction of peripheral blood there was a decrease in the level of basic fibroblast growth factor (FGFb) by 1.78 times on average (Table 5). The average amount of basic fibroblast growth factor (FGFb) in the blood serum of patients who received transfusions of mononuclear fraction of peripheral blood exceeded the average value of this indicator in the group of young healthy blood donors prior to transfusion by 2.7 times (Table 6). After completion of the cycle of transfusions of mononuclear fraction of peripheral blood, a comparison of the average values of basic fibroblast growth factor (FGFb) among patients of the main and control groups was not statistically significant (Table 7). Furthermore, among patients receiving transfusions of mononuclear fraction of peripheral blood, following completion of the cycle of 4–7 transfusions, there was a tendency towards a decrease in the levels of human vascular endothelial growth factor A (VEGF-A) and human epidermal growth factor (human EGF). Of them, in 2 patients the level of human vascular endothelial growth factor A (VEGF-A) decreased by 1.48 times on average (from 209.1 ± 4,5 pg/ml to 140.8 ± 48,6 pg/ml), and in 3 patients the level of human epidermal growth factor (human EGF) decreased by 4.12 times on average (from 146.6 ± 84.3 pg/ml to 35.5 ± 15.1 pg/ml) (Table 9). A reduction in the levels of cell growth factors naturally resulted in all 4 patients to a reduced expression of p53 by 6.02 times on average in the buccal epithelium, and in 3 of them to a reduced expression of Bcl-2 by 60.0 times on average. Restoration of the number of Leydig cells and the patient's own testosterone production in patients over 40 years old, observed during transfusion of mononuclear fraction of peripheral blood from young donors 18-23 years old [8], resulted, among patients of the main group of males (3 patients), in a reduced expression of androgen receptors (AR) by 2.12 times

on average (Table 5). All four patients 60 – 82 years old showed a thickening of the epithelial layer due to a more pronounced development of the basal and parabasal layers, an increase in the number of small blood vessels, a disappearance of parakeratosis phenomena, a disappearance or a reduction of dystrophic-modified epithelial cells (cells with optical empty cytoplasm), and an increase of lymphocytic infiltration after completion of transfusion of mononuclear fraction of peripheral blood from donors 19–23 years old, upon histological examination of the epithelium of human buccal mucosa.

Among vertebrate populations with differentiated cells subject to renewal, there is continuous destruction of old cells and their replacement by new cells. The cell renewal can occur by simple division to form two daughter cells of the same type, or through precursor cells of cambial zones. Cambial cells form progeny during division, some of which continue to differentiate, while other progeny remain poorly differentiated [9]. Committed progenitors and differentiated cells, having embarked on the path of differentiation or having completed it, can divide a limited number of times [9] and are unable to ensure tissue regeneration throughout the entire ontogeny [4]. Tissue renewal over such a long time period is impossible without the participation of a specialized system responsible for regeneration. A part of this system are pluripotent stem cells that can migrate and differentiate into all types of somatic cells and into a line of germ cells, as well as have the ability to self-renew throughout the life of the organism [4]. The basal membrane, underlying the epithelium layer, does not prevent migration of stem cells to replenish the pool of poorly differentiated cells of the cambial zone, ensuring the replacement of old cells [9]. Pluripotent stem cells are a separate branch of the differentiation of embryonic cells [9], providing for regeneration of all body tissues of the organism throughout ontogeny [4].

Old cells feature desialation of the cell surface and infringement of protection of terminal membrane glycoproteins containing mannose. The emergence of free mannose on the surface of cells makes them available for recognition by macrophages. The first line of immune defense – inflammation – begins as a reaction of natural immunity based on a phylogenetically more ancient defensive process. During necrosis (apoptosis) of old cells, and the simultaneous macrophage inflammation, as well as the surrounding epithelial and endothelial cells, the stromal cells of hematopoietic and lymphoid organs form colony-stimulating and cell growth factors, interleukins [10, 11]. Colony-stimulating factors induce proliferation of pluripotent stem cells [9] for their subsequent admission to cambial areas or directly to the place of cell death of old cells [4]. Cell growth factors, acting in various combinations, selectively stimulate the proliferation and differentiation of progenitor cells of cambial zones [9]. Management of the processes of cell differentiation is carried out by a corresponding part of the program of development, initiating the local formation of cell growth factors by the cellular environment in a strict sequence, as well as the emergence of complementary receptors in precursor cells. Incretion of cell growth factors continues until the full restoration of damaged tissue or the formation of fibrous tissue at the injury site by fibroblasts activated by the growth factors. When there are sufficient numbers in the pool of pluripotent stem cells, such as in young people, the replenishment by pluripotent stem cells of cellular composition of cambial zones and the subsequent regeneration of tissues adequately counters the death of old cells. After the replacement of old dead cells with new cells, local production of cell growth factors and colony-stimulating factors is terminated. Short-term physiological formation of cell growth factors does not lead to malignant transformation of tissues [4].

Management of stem cell migration is conducted through the formation in several stages of tissue-specific receptors. Initially, in response to desialation and the appearance on the surface of old or intensively proliferating cells of glycoproteins with free end mannose, there is binding of antigen-presenting cells (macrophages and other) with tissue specific antigens. Antigen-presenting cells deliver tissue-specific antigens of old dead cells through lymph nodes or other lymphoid organs to T-helpers [4, 11]. After analyzing the received antigens, T-helpers use antigen-presenting cells as intermediaries (increasing the likelihood of a meeting between the constantly circulating cells) to activate the pluripotent stem cells/T-killer cells with formation on their surface of tissue-specific receptors that determine the

place of their migration [4]. The presentation of antigens of virus-infected cells or antigens of foreign tissues leads to activation of killer T-cells [11], while the presentation of autoantigens of dead old cells leads to activation of pluripotent stem cells with their subsequent focus on the recovery of the respective tissues [4]. T-helper cells adhere to endothelial cells of postcapillary venules, squeeze between them, and then migrate to lymphatic vessels, through which they enter the lymph nodes. This path is repeated by T-killer cells [9], and pluripotent stem cells [4]. Activation by T-helpers of killer T-cells is accompanied by the formation of paracrine and autocrine IL-2, initiating the expression of Bcl-2, and protecting activated killer T-cells from apoptosis [11]. Similarly, after contact with T-helper cells through the expression of Bcl-2 the development of apoptosis is prevented in stem cells that are highly sensitive to adverse environmental conditions, migrating to the damaged area, or the place of death of old cells, and which are under the influence of high-level products (active forms of nitrogen and oxygen, TNFα, INFγ and others) resulting during inflammation [4]. Additionally, the expression of Bcl-2 among committed stem cells is caused by IL-7, produced by endothelial cells, between which stem cells migrate. Participation of T-helper cells in the process of tissue renewal [4] determines the high prevalence of self-antigens (99%) among the peptides presented to T-helper cells and analyzed by them, as well as the significant prevalence of subpopulations of $CD4^+$ lymphocytes (T-helpers) over $CD8^+$ lymphocytes (T-killer cells) in the blood and in the lymph [11]. Accordingly, the management of regeneration processes is the leading function of the immune system [4]. The age involution of the thymus is accompanied by a reduction in its weight, and by substitution of the epithelial compartment of the connective tissue and by adipocytes – derivatives of fibroblasts. After 50-60 years of age, there is a decrease in T-helper cells in the blood, which adversely affects the formation of tissue-specific receptors on stem cells and the regeneration process. Despite this, throughout a person's life, stem cells continue to enter the thymus, and mature T-cells continue to migrate from the thymus [11].

The death of old cells (necrosis, apoptosis) and the subsequent regeneration takes place throughout ontogeny, as a manifestation of normal functioning of the body [10, 11]. There is necrosis/apoptosis of millions of old cells every second in the body, leading to a variety of local inflammation sites. The same number of new cells must be formed in order to maintain the normal state of the body [9]. The idea of immortality of pluripotent stem cells – their ability to make an unlimited number of divisions – is arbitrary. [9] After 35-40 years of age, the abundance of the pool of pluripotent stem cells and the cambial zones they replenish is progressively reduced [1], making it impossible for old dead cells to be replaced by an adequate number of poorly differentiated progenitor cells (or stem cells that migrate directly) [4]. The formation of cell growth factors occurs in inverse proportion to the density of the cell population [9]. For this reason, in response to the destruction of old cells and the lack of their replenishment by young cells, there is increased production by epithelial and endothelial cells, and macrophages of cell growth factors (for stimulating proliferation of poorly differentiated cells of cambial zones) and colony-stimulating factors (for stimulating proliferation of pluripotent stem cells) [4]. Despite the development of these compensatory reactions in people after 35-40 years of age, the pool of pluripotent stem cells and the cellular composition of cambial zones continue to decline, managing less and less to replace old dead cells. Naturally, with increasing age, production of cellular growth factors aimed at stimulation of cell proliferation and an increase in the number of cells of cambial zones increases. The contents of cell growth factors in the blood and tissues becomes chronically high. Excessive mitogenic stimulation, increasing proportionally to age, observed in all tissues in people older than 35-40 years old, inevitably leads to metaplasia and then to malignancy [4]. The risk of malignant transformation is further increased in the presence of predisposing hereditary factors, as well as under local action of mitogenic factors (both externally and internally generated, such as, for example, under chronic inflammation) [4].

A long-term chronic inflammatory process can lead to malignancy. Prolonged duration of alteration factors, the death of a large number of cells caused by these factors, the reciprocal formation of cell growth factors designed to stimulate the proliferation of cambial cells, as well as the blocking of apoptosis

through the expression of Bcl-2 are the main pathogenetic factors of malignant transformation during the chronic inflammatory process [4]. Malignant change of chronic ulcers of the stomach, the oral cavity (with chronic occlusion) and other examples [3] support this conclusion. [4]

The risk of malignant transformation of cells with receptors of sex hormones further increases at the time of an age-related decrease in production of sex hormones necessary for cell division and differentiation of these cells. In response to the decrease in production of testosterone in men after the age of 35-40 years old, compensatory-adaptive reactions develop which are aimed at increasing mitogenic stimulation. The expression of these reactions is proportional to the degree of reduction in testosterone production [12, 13, 14, 15]. For this reason, prostate cancer is one of the most common types of cancer in men over 40 years old. The primary tumor is often androgen-dependent [16], since the malignant transformation of cells begins at an androgen-dependent stage of differentiation [14].

The increase in mitogenic stimulation is accompanied by the development of a constantly increased expression of genes responsible for the formation of cell growth factors and their receptors. There is transformation of proto-oncogenes, responsible under normal conditions for cell division in response to growth factors, into oncogenes. For example, the oncogene erbB starts to encode the abridged version of epidermal growth factor receptor (EGF), which loses the EGF-binding outer domain, but retains an intracellular domain with tyrosine-specific protein kinase activity. Cells with such defective receptors behave as if a signal for proliferation from epidermal growth factor is constantly acting on them [9].

Basic fibroblast growth factor (FGFb), epidermal growth factor (EGF), insulin-like growth factors I and II (IGF-I, IGF-II), and a number of other cell growth factors have pronounced mitogenic activity and are promoter factors in carcinogenesis [17]. Basic fibroblast growth factor (FGFb) has the greatest mitogenic activity. Constantly-increased levels of cell growth factors, blockage of apoptosis (due to expression of the protein Bcl-2) among stem cells activated by T-helpers and cambial zone cells that they replenish, and transformation of protooncogenes to oncogenes can lead to metaplasia, and subsequently to malignancy [4]. Metaplasia is indirect, and begins with proliferation of cambial cells, changed under the influence of the above-mentioned mitogenic factors, differentiated into a new cell type (e.g., into keratinizing squamous epithelium, instead of prismatic) [10]. Prolonged stimulation of mitogenic activity causes malignant degeneration of the epithelium, as well as of parenchymal, stromal, and other cells. Similarly to clonal selection of lymphocytes, every changed, undifferentiated cambial cell forms a family, giving rise to metaplased cells [4]. Since the above changes develop in the majority of tissues of all people, after 40 years of age the risk of carcinogenesis increases. The appearance of a malignant tumor becomes a predefined process. The exact location of the tumor and the time of its occurrence are determined by the individual initiating and hereditary factors [4]. A.I. Strukov and V.V. Serov [10] appropriately described the possibility of cancer in any tissue and in any organ.

The severity of compensatory formation of cell growth factors in response to the incompleteness of tissue regeneration is indicated by the level of basic fibroblast growth factor (FGFb) 5.5 times higher than the average level in the blood of patients 54 - 76 year olds with cancer of the prostate, bladder and kidney in stage III - IV of the disease in the main group (before the start of chemotherapy/targeted therapy) as compared to the same figure in young, apparently healthy blood donors from the control group. These changes have become one of the main causes of malignancy among the observed cancer patients (Table 1).

Stimulation of division of malignant cells is inadequate to ensure the progression of tumor growth. There must be constant replenishment of the cambial zone with changed cambial cells (metaplased or cancerous) with stem cells, transforming into them under the influence of the cellular environment. Data gathered by a series of authors [9, 10] on the beginning of intensive growth of tumors after the onset of its vascularization, accompanied by a significant increase in the entry of stem cells to this area, and the detection of stem cells and progenitor cells among the cells of cancerous tumors supports this conclusion [4]. Malignant transformation of cambial cells, increasing the number of their

divisions, is aimed at compensating for chronic insufficiency of cambial cells, as well as stem cells replenishing them in people older than 35-40 years [4]. This conclusion affirms the similarity of cancer cells with stem cells, for example, the appearance among them of embryonic antigens [10].

Table 1. The average content of basic fibroblast growth factor (FGFb) in the blood of cancer patients before chemotherapy/targeted therapy and in the group of young, apparently healthy blood donors

Research groups \ Indicators	FGFb, pg/ml
The main group of patients with cancer of the prostate, bladder and kidney in stage III - IV of the disease before chemotherapy/targeted therapy (n = 11)	5.0 ± 2.6
Control group of young, apparently healthy blood donors (n = 11)	0.9 ± 0.8
t	4.917
p	p < 0.001

Note: FGFb - basic fibroblast growth factor.

The examined factors, which develop during the death of old cells and the inflammation that develops concurrently, lead to the initiation of malignant transformation of cells and determine the mechanisms of progression of tumor growth. [4] On the periphery of the tumor is a zone of perifocal (demarcation) inflammation. Among other cells in this zone are macrophages [10, 18], which are drawn by free mannose of the desialation surface of intensively proliferating cells and other antigens produced during necrosis/apoptosis of cells [4]. Like osteoclasts (generated from monocytes and being a variety macrophages) that destroy bone matrix during bone turnover [9], macrophages of the zone of perifocal inflammation, releasing hydrolytic enzymes, lyse the surrounding tissues (destroy endothelium, basement membranes, fibronectin, collagen, elastin, bone matrix and other structures) to make room for tumorous cells. Intense formation of cell growth factors that stimulate the proliferation of endothelial cells (vascular growth factor and others secreted by macrophages), results in the proliferation of endothelial cells and the formation of new blood vessels (angiogenesis). In turn, the endothelium, under inflammatory conditions, produces basic fibroblast growth factor and platelet-derived growth factor, which enhance proliferation, and also forms IL-7, which causes the expression of Bcl-2. The expression of Bcl-2, increasing resistance to cell death through the apoptosis mechanism [11], screens the endothelium, migrating stem cells, and newly formed malignant cells from the resulting highly cytotoxic products of cells and macrophages [4].

Confirmation that the main reason for the increase of mitogenic stimulation and the increase in the formation of cell growth factors in people over 35-40 years old is the incompleteness of tissue renewal (incompleteness of regeneration), caused by the reduction in the pool of stem cells, comes from the increased production of basic fibroblast growth factor (FGFb) 1 month after the start of chemotherapy or targeted therapy for all cancer patients observed. On the background of developing leukopenia, indicating the suppression of the pool of stem cells by chemotherapy drugs/targeted therapy and worsening disorders of tissue renewal (violation of regeneration) in all 11 patients of the main group of the first phase of the study, who had initially elevated levels of basic fibroblast growth factor (FGFb) in the blood, a further increase in formation of this factor occurred in the blood by an average of 1.74 times (Table 2). Furthermore, among the main group of cancer patients, after chemotherapy/targeted therapy there was a tendency towards an increase of levels of human vascular endothelial growth factor (VEGF-A) and human epidermal growth factor (human EGF) in the blood. In 4 of these patients the increase in the level of human vascular endothelial growth factor (VEGF-A) averaged 1.25 times, and in 3 of them there was an increase in human epidermal growth factor (human EGF) of 1.13 times on average (Tables 3-4).

Thus, the cytostatic effect not only on the tumor, but also on the pluripotent stem cells of the bone marrow, which takes place when conducting chemotherapy or targeted therapy, leads to more stimulation of mitogenic activity, and an increased

risk of tumor recurrence and the risk of its metastasis, as well as to the appearance of new tumors in other localities. Pathological processes similar to those described above are typical of patients with rheumatological diseases, treated with immune-suppressive therapy, as well as for all people over 35-40 years old, who have a reduction in their pool of pluripotent stem cells and have a consequent disorder of tissue renewal. Both groups have an increased risk of malignant diseases.

Table 2. Average content of basic fibroblast growth factor (FGFb) in the blood in cancer patients before and one month after the start of chemotherapy/targeted therapy

Research groups\Indicators	FGFb, pg/ml
The main group of patients with cancer of the prostate, bladder and kidney in stage III-IV of the disease before chemotherapy/targeted therapy (n = 11)	5.0 ± 2.6
The main group of patients with cancer of the prostate, bladder and kidney in stage III - IV of the disease one month after the start of chemotherapy/targeted therapy (n = 11)	8.7 ± 4.7
t	3.607
p	$p < 0.005$

Note: FGFb – basic fibroblast growth factor.

On the surface of the activated endothelium, platelets, and white blood cells, one can observe expression and adhesion of coagulation factors leading to fibrin formation [9, 18]. Adaptive changes in the endothelium in the area of inflammation are accompanied by intra- and extravascular clotting of fibrinogen and the formation of blood clots. Necrosis develops as a result of thrombosis of the blood vessels around the inflamed tissue area. Accordingly, tumors are often subject necrosis and ulceration [10]. Due to incompleteness of the regeneration process following the death of old cells, and the presence of accompanying local inflammation sites, there is a greater risk of blood clots as well in the majority of tissues in people older than 35-40 years of age [4].

During mitosis, the cells are rounded and lose their strong bond with each other (due to a reduction in cell adhesion and loss of cell-cell contacts). The integrity of the tissue consisting of such cells is disrupted [9]. For this reason, metastasis begins faster in tumors with the highest intensity of division of cancer cells [4]. Malignant cells, the formation of which is directed at fulfilling a deficit of pluripotent stem cells, acquire a similarity to these cells (e.g., embryonic antigens appear on these cells). During metastasis, malignant cells repeated the path and mechanisms of migration of stem cells in tissue regeneration. By analogy to the pluripotent stem cells, in order for the malignant cells to have contact with T-helper cells, and for the formation in them of tissue-specific receptors, these cells must be continuously circulated through the secondary lymphoid organs. Tumor cells attach to endothelial cells of postcapillary venules, squeeze between them, and flow through the lymphatic vessels to the lymph nodes and then through the corresponding groups of lymph nodes and blood vessels into the thoracic duct, through which they return to the blood. This circulation occurs constantly, leading to tumor dissemination [4]. The focus of metastasis of malignant cells is determined by the formation of the corresponding tissue-specific receptors on their surface. This is preceded by binding in secondary lymphoid organs of antigen presenting cells (carrying complexes of tissue specific antigens of dead old cells on their surface) with T-helper cells, activation of T-helper cells, and the subsequent cooperation (mediated by antigen-presenting cells) of T-helper cells with tumor cells to form tissue-specific receptors on their surface (old dead cells complimentary to tissue specific antigens) [4]. The appearance of tissue-specific “homing receptors” determines the direction of migration of malignant cells to the places of death of old cells. The predominance of the death of old cells over the regeneration process in most tissues in people older than 35-40 years old, accompanied by the appearance of an excess of tissue-specific chemoattractant (which tissue-specific antigens of dead old cells serve as), promotes, together with a reduction in adhesion and the loss of contacts between intensively proliferating cells, metastasis of malignant tumors [4]. After contact with T-helper cells and the formation of

tissue-specific receptors, cancerous cells, entering the places of death of old cells under the influence of the cellular environment, determining the direction of differentiation, change their histological structure, acquiring properties of progenitor cells of the cambial zone of the given tissues. This can create the illusion of development of multiple primary tumors. If metastases are formed only thanks to adhesive-cellular interactions, then proliferation of tumor cells will occur with a minimal influence of the cellular environment, without the tumor cells' inclusion in the mechanisms of local differentiation. The histological structure of such a tumor will largely correspond to the primary tumor.

Blocking osteoclasts (macrophages), as antigen presenting cells, under the use of bisphosphonates, disrupts the process of presenting antigens of dead old cells of the bone tissue to helper T-cells for formation of the corresponding receptors in tissue-specific stem cells and tumor cells. Accordingly, the effect of bisphosphonates is achieved by blocking the mechanism of migration of stem and tumor cells during regeneration of old dead cells of bone tissue. Violation of the natural mechanism of regenerating bone tissue with lysis by osteoclasts of dead old cells when using bisphosphonates is accompanied by sequesters, consisting of non-phagocytosed conglomerates of old dead osteocytes [4].

The above-described regularities are demonstrated by the clinical example of increased levels of basic fibroblast growth factor (FGFb), human vascular endothelial growth factor A (human VEGF-A), and human epidermal growth factor (human EGF) in the blood serum of a 57 year-old patient before and 1 month after the start of targeted therapy given to the patient for kidney cancer $T_2N_1M_1$ (Table 3). Similar changes were observed in a 54 year-old patient before and 1 month after the start of palliative chemotherapy for bladder cancer $T_2N_1M_1$ (Table 4). These examples show an increase in production of cell growth factors and an increase through them of mitogenic stimulation in response to suppression of the pool of stem cells and the consequent violation of tissue renewal (violation of regeneration). The results show that together with their effect on the tumor, chemotherapy or targeted therapy, suppressing the pool of pluripotent stem cells and disrupting tissue regeneration, lead to the stimulation of mitogenic activity, increasing the risk of a recurrence of the tumor, its metastasis and the development of new tumors in other localizations. For this reason, during a place-localized tumor process, in order to minimize toxic effects on bone marrow the pluripotent stem cells contained in this marrow, it is advisable to conduct chemotherapy/targeted therapy in a specific region with intra-arterial selective drug injections.

To normalize the process of tissue renewal, for full replacement of old cells by progenitor cells, and to reverse the pathological processes associated with violation of regeneration individuals older than 40 years old require restoration of the numerical strength of the pool of pluripotent stem cells [4]. Positive clinical dynamics in cancer patients who have been prescribed with colony stimulating factors after chemotherapy indirectly confirms this conclusion [19]. Unfortunately, the effect of stimulation using medications with colony-stimulating factors is temporary.

Table 3. Clinical example of an increase in the levels of cell growth factors in a 57 year-old patient receiving target therapy for kidney cancer $T_2N_1M_1$

Indicators\Patient	57 year-old patient, with a diagnosis of kidney cancer $T_2N_1M_1$, receiving target therapy	
	Before the start of target therapy	One month after the start of target therapy
FGFb, pg/ml	5.4	17.1
human VEGF-A, pg/ml	186.4	232.4
human EGF, pg/ml	188.1	244.6

Note: FGFb - basic fibroblast growth factor, human VEGF-A - human vascular endothelial growth factor A, human EGF - human epidermal growth factor.

Table 4. Clinical example of an increase in the levels of cell growth factors in a 54 year-old patient receiving palliative chemotherapy for bladder cancer $T_2N_1M_1$

Indicators\Patient	54 year-old patient, with a diagnosis of bladder cancer $T_2N_1M_1$, receiving palliative chemotherapy (PCT)	
	Before the start of PCT	One month after the start of PCT
FGFb, pg/ml	7.9	15.2
human VEGF-A, pg/ml	655.6	686.4
human EGF, pg/ml	243.8	250.0

Note: PCT - palliative chemotherapy, FGFb - basic fibroblast growth factor, human VEGF-A - human vascular endothelial growth factor A, human EGF - human epidermal growth factor.

The chimerism effect opens new possibilities for restoring the pool of pluripotent stem cells and restoring regeneration. Thymic epithelial cells make up the microenvironment of developing thymocytes. During direct cell contacts, they transmit T-helpers information about antigens of their own tissues, and provide them with the type of responses to the presented antigens [11]. Settling in the thymus by stem cell provides not only the consequent formation of T-cells, but also renewal of cells of the epithelial reticulum and the cortico-medullary thymic structure [11]. Considering that thymic epithelial cells fulfill the process of teaching T-helpers to differentiate their own and others' antigens, the renewal of old dead epithelial cells of the thymus by donor allogeneic stem cells, leads to the appearance of epithelial cells of the thymus, formed from the donor's stem cells. Such epithelial cells begin to teach T-helper cells of the recipient to recognize the donor's cells as "their own." After transfusion, pluripotent stem cells contained in the mononuclear fraction of peripheral blood form colonies in the bone marrow and are involved in the renewal of all body tissues, including epithelial cells of the thymus. The individual becomes a chimera. Chimerism is widely distributed in nature. For example, chimerism occurs in all females after childbirth. During childbirth, women receive a small amount of their child's blood into their bloodstream, including pluripotent stem cells contained in the blood. The latter form colonies in the mother's bone marrow [4]. During birth of children with a different blood group than their mother's blood group, the formation of the erythroid lineage of the mother is partially derived from the stem cells received from the mother's children. In multipara women, this leads to difficulties in determining their blood group.

Artificial formation of a chimeric individual through transfusion of mononuclear fraction of peripheral blood taken from young donors 18-23 old containing pluripotent stem cells can be used to maintain the normal population of the pool of pluripotent stem cells, to reinstate regeneration, and to reverse the development of regeneration disorders in people over 40 years old [4, 8]. This supposition is confirmed by the increase, on average, by 3.3 times (from 1 to 2 - 5 cells in 1 mcl, to, on average, 3.3 cells in 1 mcl) of the content of hematopoietic progenitor cells (HPC) $CD34^+$ of peripheral blood among 4 patients of the main group of the second part of the study after 3 - 6 months following the completion of a cycle of 4-7 transfusions of mononuclear fraction of peripheral blood. A comparison of the results following the instructions of the Stem-Kit Reagents, Beckman Coulter Company (France) of 117 healthy people of different age groups with a maximum value of $CD34^+$ of peripheral blood in young people at 6.5 cells/mcl and a minimal value of $CD34^+$ of peripheral blood in older age patients of 0.5 cells/mcl shows that in patients, following completion of the cycle of transfusions of mononuclear fraction of peripheral blood from donors 19-23 years old, the content of hematopoietic progenitor cells (HPC) $CD34^+$ of peripheral blood increased to a value characteristic of young people. Thus, in 4 patients being observed ranging in age from 60-82 years old, it was possible to

restore the stem cell pool, and thus the regeneration process, to the level of young adults (Tables 5, 8).

All 4 patients aged 60-82 years old, upon completion of transfusions of mononuclear fraction of peripheral blood taken from young donors, and recovery of tissue renewal (recovery of regeneration), showed, in a histological examination of mucous buccal epithelium, a regression of atrophic changes with thickening of the epithelial layer due to a more pronounced development of the basal and parabasal layers, with an increase in lymphocyte (mononuclear) infiltration (as a manifestation of increasing the number of migrated, committed stem cells) and other previously described changes. Furthermore, the choice of the buccal epithelium was random, and was conditioned by the simplicity of collecting this material. Changes in the buccal epithelium reflected general changes in tissues on the background of recovery of the regeneration process and a reduction in the amount of cell growth factors in the blood plasma of patients observed after completion of the cycle of transfusions.

Recovery of the pool of pluripotent stem cells and reconstitution of tissue regeneration (restoration of regeneration) in 4 patients, 60-82 years old, after completion of the cycle of transfusions of mononuclear fraction of peripheral blood led to a significant decrease in all patients of the content of basic fibroblast growth factor (FGFb), the average level of which decreased 1.78 times, and approached the level of the same period in 4 young people 19-22 years in the control group (Table 5). Furthermore, the mean content of basic fibroblast growth factor (FGFb) in the blood serum in 4 patients receiving transfusions of mononuclear fractions of peripheral blood, before the transfusions, was significantly higher, at 2.7 times more than the average value of this indicator in the control group of 4 young apparently healthy blood donors (Table 6). A comparison of the average values of basic fibroblast growth factor (FGFb) in patients of the main group after completion of transfusions and young people in the control group showed no statistical differences between them (Table 7).

After restoration of regeneration among 4 patients of the main group of the second part of the study, which occurred after completion of the cycle of transfusions of mononuclear fraction of peripheral blood, there was a tendency towards a decrease in the levels of human vascular endothelial growth factor A (human VEGF-A), and human epidermal growth factor (human EGF). Out of 4 patients of the main group, 2 patients had a level of human vascular endothelial growth factor A (human VEGF-A) which decreased by 1.48 times on average, while in 3 of the patients the level of human epidermal growth factor (human EGF) decreased on average by 4.12 times (Table 9).

The expression of the protein p53, as a regulator of the cell cycle and suppressor of formation of malignant tumors, as well as the expression of Bcl-2 protein, which is an intracellular regulator that prevents the development of apoptosis with the aim of compensatory preservation of the number of cellular composition of cambial zones during a deficit of entry of stem cells, increase when there is an greater mitogenic stimulation, and decrease when there is a reduction in mitogenic stimulation. Restoring the replenishment of cambial zones by migrating committed stem cells from donors, and the reciprocal reduction in the formation of cell growth factors in all 4 patients after completion of the cycle of transfusions of mononuclear fraction of peripheral blood, naturally led to a reduction in the buccal epithelium of the expression of p53 by 6.02 on average, while for 3 of the patients it led to a reduced expression of Bcl-2 by 60.0 times on average (Tables 5 and 9).

Restoration of the number of Leydig cells and their production of testosterone in patients older than 40 years old, as observed during as a result of transfusion of mononuclear fraction of peripheral blood from young donors 18-23 years old [8], resulted, among male patients of the main group (in 3 patients), in a decrease in the expression of androgen receptors (AR) by 2.12 times on average (Tables 5 and 9). When there is a lack of testosterone, the number of androgen receptors (AR) that fail in capturing hormone molecules increases. In contrast, when there is restoration of testosterone production, all newly formed androgen receptors (AR) on the core surface directly capture the testosterone molecules present in adequate numbers, and are immersed into the cell nucleus, while the expression of androgen receptors (AR) is significantly reduced [14]. This pattern was observed in the main group after the completion of their transfusion of mononuclear fraction of peripheral blood.

Table 5. Indicators among patients receiving transfusions of mononuclear fractions of peripheral blood, before and after the transfusions

Study groups\Indicators	HPC $CD34^+$, cells/mcl (n=4)	FGFb, pg/ml (n=4)	p53, % (n=4)	Bcl-2, % (n=3)	AR, Histochemical score (n=3)
Main group prior to transfusion of mononuclear fraction of peripheral blood	1 ± 0	1.9 ± 0.8	52.7 ± 25.5	60.0 ± 29.4	60 ± 29.4
Main group 3-6 months after completion of the cycle of transfusions of mononuclear fraction of peripheral blood	3.3 ± 1.1	1.1 ± 0.7	8.7 ± 8.9	0	28.3 ± 29.5
t	3.576	2.458	4.130	2.882	7.181
p	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.05$	$p < 0.001$

Note: HPC $CD34^+$ - hematopoietic progenitor cells $CD34^+$, FGFb - basic fibroblast growth factor, AR – androgen receptors.

Table 6. Average contents of basic fibroblast growth factor (FGFb) in the blood of patients who received transfusions of mononuclear fraction before the transfusions, and among the group of young, apparently healthy blood donors

Study groups\Indicators	FGFb, pg/ml
Main group of patients prior to transfusion of mononuclear fraction of peripheral blood (n = 4)	1.9 ± 0.8
Control group of young, apparently healthy blood donors (n = 4)	0.7 ± 0.6
t	2.448
p	$p < 0.05$

Note: FGFb - basic fibroblast growth factor.

Table 7. Average contents of basic fibroblast growth factor (FGFb) levels in the blood of patients who received transfusions of mononuclear fraction after the transfusions, and among the group of young, apparently healthy blood donors

Study groups\Indicators	FGFb, pg/ml
Main group of patients after transfusion of mononuclear fraction of peripheral blood (n = 4)	1.1 ± 0.7
Control group of young, apparently healthy blood donors (n = 4)	0.7 ± 0.6
t	0.734
p	$p > 0.05$

Note: FGFb - basic fibroblast growth factor.

Table 8. Reference values of hematopoietic progenitor cells (HPC) $CD34^+$ in peripheral blood of healthy individuals of various ages

	$CD34^+$ in peripheral blood of healthy individuals (cells/mcl)			
Manual method, n = 117	Minimal contents	Maximum contents	Average contents	Standard deviation
	0.50	6.50	2.36	1.14

Note: these data are given from the instructions of Stem-Kit Reagents, Beckman Coulter Company, France, section 13.1. The range of normal values was determined in blood samples from healthy subjects of various age groups (n = 117; 58 men and 59 women).

The effectiveness of regeneration in individuals older than 40 years old upon receiving transfusions of allogeneic pluripotent stem cells depends on the difference in age between the recipient and the young donor. The stage of the long-term intracellular program in which the donor cells and the recipient cells are found is of fundamental importance. The presence of long-term intracellular programs of pluripotent stem cells which determine the cells' proliferative potential (their ability to support the necessary number of cells in their own pool) significantly separates pluripotent stem cells of young people from analogous cells of people older than 35-40. The ability of stem cells to maintain quantities in their own pool decreases in proportion to age. The numerical strength of their pool after 35 years of age goes down by 1% per year, leading to systematic changes [4]. For this reason, fibrosis develops in most tissues and organs in people over 35-40 years of age at an intensity equal to the speed of the decrease in the pool of pluripotent stem cells – 1% per year [2, 4]. When there is a major difference in age between young donors and recipients over 40 years old, the proliferative potential of pluripotent stem cells of donors (their ability to maintain the number of own pool) is higher than the proliferative potential of the recipient. In response to the formation of colony-stimulating growth factors, this leads to the dominance of transfused and formed colonies in the bone marrow of the donor's pluripotent stem cells over the analogous cells of recipients when renewing all of their tissues. The latter occurs mainly due to the donors' stem cells [4, 8].

Transfusion of pluripotent stem cells doesn't lead to their rejection by the immune system of the recipient, since stem cells have a suppressed expression of all tissue-specific antigens, except for HLA-G [9, 20]. The subsequent development of immune tolerance is caused by the formation of chimerism, preventing rejection of differentiated cells of the recipient formed from the donor's stem cells. Renewal of the recipient's tissues by cells from a donor of the opposite sex is accompanied by violation of the formation of sex hormones and infertility. Erythroid lineage of hematopoiesis is formed, among other things, during differentiation of donor pluripotent stem cells that form colonies in the bone marrow of the recipient. Transfusion of peripheral blood stem cells from a donor with a different blood group than the recipient's blood group will lead to the formation of red blood cells with the donor's antigens, and to the development of incompatibility reactions [4, 8].

It follows from the above-said that the transfusion of pluripotent stem cells as part of mononuclear fraction of peripheral blood with the aim of renewing the pool of pluripotent stem cells in recipients older than 40 years of age should come from young donors 18-23 years old of the same sex and blood groups as the recipient (RF patent number 2350340). In order to reduce the antigenic load, it is expedient for all transfusions to each of the recipients to come from a single donor. Since there are small amounts of antigens of other blood groups in multiparous women under normal conditions, which does not lead to any health complications, it is enough to select donors taking into account the blood groups, the antigens of which have strong immunogenic properties. Transfusions of mononuclear fraction of peripheral blood are required to be carried out multiple times before the restoration of the numerical strength of the recipients' pool of pluripotent stem cells is complete. Accordingly, in order to obtain a systemic effect, there must be repeated procurement of mononuclear fraction of peripheral blood from a single donor (for each recipient), which limits the choice of donors to the minimally allowed age of 18 years old [4, 8].

The effectiveness of therapy aimed at restarting regeneration of tissues (recovery of regeneration) is demonstrated by the clinical example of a 60 year-old patient, who after receiving 7 transfusions of mononuclear fraction of peripheral blood, procured from one 20 year-old donor, showed a five-time increase in the contents of hematopoietic progenitor cells $CD34^+$ of peripheral blood, which led to the recovery of cell regeneration, and to a regular decrease of FGFb in the blood by 5.3 times, of human VEGF-A by 1.1 times, and of human EGF by 1.1 times. At the same time as there was a decrease in the levels of cell growth factors in the buccal epithelium, the patient also showed a decrease in the expression of p53 by 4.0 times, and of Bcl-2 by 70.0 times. The decrease in the expression of androgen receptors (AR) by 6.0 times in the buccal epithelium gave proof of a recovery in the numbers of Leydig cells and of an increase in testosterone production by these cells. The

data received speak of a significant decrease in the risk of carcinogenesis in the patient. Thanks to the formation of a chimerism as a result of transfusions of mononuclear fraction of peripheral blood, the patient's immune system did not react by rejecting the transfused cells. In particular, there was no increase in ESR. On the contrary, ESR had a tendency to decrease, since recovery of the process of tissue regeneration in the patient was accompanied by a decrease in mitogenic activity, which in turn led to a decreased reaction by the patient's immune system to the proliferating cells (Table 9).

Table 9. Clinical example of a 60 year-old patient who received 7 transfusions of mononuclear fraction of peripheral blood from a 20 year-old donor

Indicators of the 60 year-old patient	Before the beginning of transfusions	After completion of the cycle of 7 transfusions, 3 months after the last transfusion
Hematopoietic progenitor cells $CD34^+$, cells/mcl	1	5
FGFb, pg/ml	2.1	0.4
VEGF-A, pg/ml	213.6	189.5
EGF, pg/ml	38.9	35.5
p53, %	60	15
Bcl-2, %	70	0
AR, Histochemical score	30	5
ESR, mm/hour	6	3

Note: FGFb – basic fibroblast growth factor, human VEGF-A – human vascular endothelial growth factor A, human EGF – human epidermal growth factor, AR – androgen receptors.

Conclusion

A reduction in the pool of pluripotent stem cells and the consequent violation of tissue renewal in people older than 35-40 years old are the main reasons for an increase in mitogenic activity, which leads to an increased risk of carcinogenesis. Transfusion of mononuclear fraction of peripheral blood procured from young donors 18-23 years old with the same blood groups and sex as the recipient (RF patent number 2350340), allows people over 40 years old to reestablish the pool of pluripotent stem cells and the process of tissue renewal, reduce mitogenic stimulation and the risk of cancer, improve the results of cancer treatment (aimed at removing or suppressing the growth of cancer cells), and can also be seen as a promising way to reduce biological age, while providing a significant prolongation of life and the ability to work (while maintaining a high quality of life).

References

[1] Teplyashin AS, Korzhikova SV, Sharifullina SZ, Chupikova NI, Rostovskaya MS, Savchenkova IP. Characteristics of human mesenchymal stem cells isolated from bone marrow and adipose tissue. *Tsitologiya* 2005; 47(2): 130-135.

[2] Tareeva IE. Nephrology. *Meditsina* (*Moscow)*. 1995; 1: 270-303.

[3] Napalkov NP. General oncology. *Meditsina (Leningrad)*. 1989: 9-28.

[4] Pechersky AV, Pechersky VI, Aseev MV, Droblenkov AV, Semiglazov VF. Several aspects of the regeneration process carried out by means of pluripotent stem cells. *Tsitologiya* 2008; 50(6): 511-520.

[5] Merkulov GA. Course in histopathologic technology. *MedGiz (Leningrad)*. 1961: 1-346.

[6] Jonat W, Maass H, Stegner HE. Immunohistochemical measurement of estrogen receptors in breast cancer tissue samples. *Cancer Res* 1986; 46: 4296-4298.

[7] Glantz SA. Primer of biostatistics. *Practica (Moscow)*. 1999: 27-121, 285-322.

[8] Pechersky AV, Pechersky VI, Smolyaninov AB, Vilyaninov VN, Adylov ShF, Semiglazov VF. Implementing cellular technologies to restore the regeneration process in people of older age groups.

Bulletin of the North-Western State Medical University named after I.I. Mechnikov 2014; 6(4): 52-62.

[9] Alberts B, Bray D, Lewis J, Raff M, Roberts K, Watson JD. Molecular biology of the cell. *Mir (Moscow)*. 1994; 2: 176-529, 3: 7-381.

[10] Strukov AI, Serov VV. Pathological Anatomy. *Meditsina (Moscow)*. 1993: 47-262, 453-458.

[11] Yarilin AA. Fundamentals of Immunology. *Meditsina (Moscow)*. 1999: 17-440.

[12] Pechersky AV, Semiglazov VF, Mazurov VI, Karpischenko AI, Mikhailichenko VV, Udintsev AV. Androgen administration in middle-aged and ageing men: effects of oral testosterone undecanoate on dihydrotestosterone, estradiol and prostate volume. *International Journal of Andrology* 2002; 25: 119-125.

[13] Pechersky AV, Semiglazov VF, Loran OB, Mazurov VI, Karpishchenko VF, Nikiforov VF, Kalinina NM, Drygina LB, Davydova NI, Skorobogatykh MG. Changes in cytokine levels in patients with prostate cancer after orchiectomy. *Laboratory diagnostics* 2003; 2: 26-30.

[14] Pechersky AV, Semiglazov VF, Komyakov BK, Guliyev BG, Gorelov AI, Novikov AI, Pechersky VI, Simonov NN, Gulyayev AV, Samusenko IA, Vonsky MS, Mittenberg AG, Loran OB. Changes in the expression of steroid hormone receptors during development of partial androgen deficiency (PADAM). *Tsitologiya* 2005; 47(4): 311-317.

[15] Pechersky AV, Semiglazov VF, Mazurov VI, Karpishchenko AI, Pechersky VI, Zybina NN, Davydova NI, Kravtsov VYu, Proshin SN, Skorobogatykh MG, Loran OB. The influence of partial androgen deficiency of aging men on the development of metabolic syndrome. *Laboratory Diagnostics* 2006; 4: 12-19.

[16] Moiseenko VM, Urmancheeva AF, Hanson KP. Lectures on basic and clinical oncology. *N-L Publishing House (St. Petersburg)*. 2004: 369-430.

[17] Bershtein LM. Hormonal carcinogenesis. *Science (St. Petersburg)*. 2000: 10-129.

[18] Serov VV, Paukov VS. Inflammation. *Meditsina (Moscow)*. 1995: 62-261.

[19] Konstantinova MM. Principles and methods for evaluating the effectiveness of drug therapy and quality of life of patients with malignant tumors. In Lectures on basic and clinical oncology ed by Moiseenko VM, Urmancheeva AF, Hanson KP. *N-L Publishing House (St. Petersburg)*. 2004: 573-597.

[20] Roitt I, Brostoff J, Male D. Immunology. *Mir (Moscow)*. 2000: 63-64, 168-193.