Brief analytical report presenting findings of scientific research and developments for 2016

1. 'Molecular, genetic and cellular mechanisms for the implementation of abnormal processes within a human body in the presence of blood disorders' (fundamental).

The most significant results obtained

Section: 'Molecular and genetic testing of hemophilia A and B in Russia'

In 2016, the number of patients with hemophilia A, for whom mutations in the factor VIII gene were determined, significantly increased. To date, mutations were found mainly in 128 unrelated patients with a severe disease. Common inv22 inversion was found in 70 of 139 examined patients (50%). In any case of sporadic hemophilia (de novo mutation, first patient in the family), proband mothers carried this genetic defect. Comparison with data of family analysis of polymorphic markers has shown that in these families proband commonly inherits a healthy grandfather's factor VIII gene. This coincides with the existing idea stating that inv22 inversion occurs de novo more frequently in spermatogenesis than in oogenesis. A more rare inv1 inversion is found in 5 of 242 patients (2.1%). Non-inversion mutations were detected in 53 patients. In total, 48 different genetic defects were found. 28 of them are new and have never occurred in the global population previously. Missense mutations (25.52%) and nonsense mutations (9.19%) predominate among the found disturbances. C.3637delA mutation is the most common as it was found among 5 unrelated patients. 4 other mutations (c.4379_4380insA, His634Arg, His2026Arg, Arg2228Gln) were found twice. Other mutations were unique and seen in single patients. Two large-scale deletions of the factor VIII gene, del ex6 and del ex7-12 obtain a total characteristic with determined break points. The length of del ex6 is 3,748 pn, the length of del ex7-12 is 29,960 pn. Mutations in the factor VIII gene were identified in two women with clinical signs of hemophilia A. In addition to what was previously stated, our research was expanded in 2016. It included rare coagulopathies such as different variants of fibrinogenemia (afibrinogenemia, disfibrinogenemia, hypofibrinogenemia), deficiency of the factor VII gene and factor XIII gene (Hageman factor disease). Mutations were found within a fibrinogen gene (FGA, FGB or FGG) of 4 patients with various forms of fibrinogenemia, gene FVII mutations in 4 patients with factor VII deficiency and gene FXII mutation in 4 patients with Hageman factor disease. Novel mutations included CD66 delT in the FGA gene, replacement of CD222 AAG-AAA in the FGG gene that disturbs splicing, missens mutation Gln283Arg in the FGB gene and missens mutation Gly22Arg in the FVII gene. Diagnostic practice of hemophilia A carriage was performed for women from 22 families, including 2 prenatal DNA diagnostic tests. In the majority of cases, two parallel approaches were used such as indirect diagnostics of polymorphic markers and direct diagnostics with determination of mutations in the VIII gene.

Section: 'Role of clonal T-cell component in pathogenesis of B-cell diseases'

A testing of clonality for H chain genes of IGH immunoglobulin (VH-JH, FRI/ FRII/FRIII), beta chains of TCRB T-cell receptor (Vβ-Jβ/ Dβ-Jβ), and gamma chains of TCRG T-cell receptor (Vγ-Jγ) was conducted among 8 patients diagnosed with rheumatoid arthritis (RA) and 6 patients diagnosed with systemic lupus erythematosus (SLE). Polyclonal IGH gene rearrangements were detected in all of them. In most cases of rheumatoid arthritis and systemic lupus erythematosus, a poly- (in 4 out of 14 patients) or oligoclonal (in 7 out of 14 patients) pattern of rearrangements of the TCRG and TCRB genes was found. Monoclonality of T-cells in the general population, i.e. presence of the dominating clonal product, was found in 3 patients of 14 (21%). To understand the immunotype of these cellular clones, cells of 7 patients were selected (5 PA and 2 CKB).

Patients with oligo- and monoclonality within the general population of T-cells were mainly used for selection purposes. Two populations of T-cells such as CD4+ and CD8+ were isolated from peripheral blood leukocytes. Testing of clonality among these T-cells has shown that clonal T-cells were found both in CD4+, and CD8+ of T-cell population. In either case no isolated monoclonal peaks were detected for CD8+ lymphocytes. This was characteristic of autoimmune hemolytic anemia (AHA) and previously displayed. The difference was statistically significant for patients with AHA ($p < 0.0001$).

Section: 'Biophysical properties of erythrocytes in patients with disturbed erythropoiesis'

Dynamic density distribution range of erythrocytes was tested in 37°C during RBC incubation. It was shown that decreased erythrocyte density was observed in healthy volunteers and majority of patients with various blood diseases following daily incubation in 37°C. This occurred due to higher red blood cell count affected by decreased blood pH as a consequence of lactate accumulation. 2 days of incubation were followed by an increased density of red cells. The reason for that is activation of the Gardos channels induced by an increased level of intracellular calcium ions associated with metabolic starvation of red cells. This conclusion was supported by a testing of dynamic density distribution range of erythrocytes in the presence of propranolol activator of the Gardos channel. In 4 days of incubation, fractions of red cells with a decreased density (postlytic) were detected and red cell hemolysis was recorded. The results of dynamic density distribution range of erythrocytes in physiological temperature made it possible to select patients with accelerated metabolic starvation of red cells. These were mainly patients with thalassemia and some patients with AHA. In patients with thalassemia, an accelerated increase in the density of red blood cells is observed due to an initially reduced level of ATP in red blood cells. Therefore, they are destroyed faster than healthy red blood cells in the spleen, which leads to its increase.

New results that display the effect of red cell population properties on hemostasis were obtained. A positive correlation between the extent of intraoperative blood loss (IBL) during knee replacement in patients with hemophilia A, HCT over 38.5% and average density of red cells was detected.

It has been shown for the first time that inclusion of red cells into a blood clot depends both on hematocrit and red cell density. In a low HCT and high red cell density, blood clot HCT (RBC volume ratio within a blood clot) is decreased.

According to the preliminary results, anticoagulant therapy with non-fractioned heparin and xarelto decreases inclusion of RBC into a blood clot, whereas Clexane, Thrombo ASS and Clopidogrel produce almost no effect on the properties of a blood clot.

Red cell density distribution test method is published and used at the Hematological Research Center of the Ministry of Health of the Russian Federation to assess the properties of RBC population. The test results were published and reported during scientific conferences and seminars. The analysis method of blood clot properties is under development. It is planned to be implemented as an additional criterion assessing the reasons for thrombosis and haemorrhagias and selecting optimal correction methods of haemostatic disturbances.

Section: 'Examining the three-dimensional dynamics of blood coagulation'

In vitro tests were conducted to search for a qualitative effect of thiol-containing isomerases on the biochemical cascade of blood coagulation. During experiments it was found that the nature of the coagulation processes is not changed in response to the inhibition of thiol-containing isomerases.

A series of experiments to grow thrombi under convection-enhanced conditions were conducted. It was found out that the method of forming thrombi under the flow is not subject to standardization.

Thus, the in-vitro method of forming thrombi on the inpatient basis was developed. It was subsequently transferred onto a pilot unit to examine the processes of fibrinolysis under hydrodynamic conditions.

2. 'Studying immune modulating properties of multipotent mesenchymal stromal cells (MMSC) to improve therapeutic effectiveness in patients after allogeneic stem cell transplantation' (fundamental)

The most significant results obtained

The level of gene expression to MMSC and fibroblast colony-forming units (FCFU) was studied among patients with hemoblastoses and healthy donors. The relative expression level of genes responsible for self-support (FGFR1, FGFR2, PDGFRA, PDGFRB), proliferation (FGF2), morphogenesis (BMP4, SOX9, SPP1, BGLAP, PPARG, VEGF), hematopoiesis regulation (Jag1, TGFB2, TGFB1, CSF, ANG1) and immune response (IL1b, IL1R, IL6, IL8) was estimated.

All MMSC obtained from bone marrow samples of patients did not differ morphologically from those taken from healthy donors. In the study, an increased time of the first MMSC passage was detected among patients with hemoblastoses as compared to healthy donors and a decreased aggregate 3-passage cellular production was found among patients with AML. This could be due to a smaller number of stromal precursors or their disturbed proliferation associated with total expansion of blast cells suppressing normal hematopoiesis. It was also shown that a higher gene expression at onset promoted proliferation of leukemia cells and genes inhibiting MMSC proliferation and migration. Therapy restored concentration of genes responsible for MMSC differentiation. In particular, expression of IL-6, IL1b1, CSF, VCAM, IGF1 genes was restored. At the same time, during therapy, an increase in the expression level of the PDGFRa gene encoding a protein that is a receptor for the growth factor — PDGFa was noted. PDGFRa is a powerful mitogen for cells of mesenchymal origin. It plays an important role in angiogenesis, and also has the ability to maintain hematopoiesis. An increase in its expression during therapy indicates the restoration of the microenvironment. Expression of genes responsible for MMSC morphogenesis has been studied: osteocalcin (BGLAP), SOX9, with a significant decrease in the level of gene expression at the onset of the disease and a gradual increase during therapy.

Changes in MMSC population activated with gamma interferon (IFN) were examined. Intramedullary human MMSC were cultivated under regular conditions when different concentrations of IFN were added to the culture medium. It was shown that aggregate MMSC production significantly decreased in the long-term cultivation in the presence of IFN, whereas 4-hour effect produced no influence on cells. After 4 hours of cultivation, the expression level of ID1 increases by 300 times, CSF1 by 7, and IL6 by 2.4 times, which significantly increases the immunomodulatory properties of MMSCs. As MMSCs were cultured after IFN treatment, a significant increase in the expression of HLA-ABC and HLA-DR was observed for 4 hours. When using MMSCs from one's own hematopoietic stem cell donor, an increase in HLA-DR expression has no immunological significance. The analysis of the activation of MMSCs showed that their treatment of cells in the used doses for 4 hours does not significantly affect cell production for 4 days.

Changes in MMSCs were found while examining inhibited proliferation of T-cell within MMSCs of donors after interaction with allogenic T-cells. Addition of activated T-cells (cultivation in the presence of PHA) resulted in reduction of cells irrespective of preliminary IFN treatment. In co-cultivation with activated T-cells, the concentration of live cells was not decreased in preliminary IFN treated cultures. It is indicative of cell sensibilization towards the factors produced by activated T-cells. Thus, for the first time, in the in vitro system, results were obtained indicating significant changes in the properties of MMSCs when they enter the body, where these cells interact with lymphocytes in the bloodstream and in tissues.

Differences in the ability of MMSCs to inhibit proliferation of allogenic and syngeneic T-cells triggered for division were estimated with the method of T-cell Carboxyfluorescein succinimidyl ester (CFSE) staining. No differences in the inhibition of proliferation of activated syngeneic and allogeneic T-cells were recorded during preliminary experiments. With allogeneic T-cells, proliferation was decreased by 2.2 ± 0.6 times; with syngeneic cells, it was decreased by 2.0 ± 0.5 times.

Immunomodulating properties of MMSCs were examined to prevent acute GVHD following allogeneic transplantation of hematopoietic stem cells. Administration of MMSCs decreased development of GVHD among patients with related donors by two times as compared to patients who were not administered MMSCs. The prevention was not effective in 13.5% of cases. A significant decrease of aggregate cellular production and relative expression of CFH, FGFR1, PDGFRa and ICAM1 was detected in non-effective samples of MMSCs. The obtained data were used for the first time to display possible mechanisms of protection against GVHD. Decreased expression of complement factor-H (CFH) is associated with decreased cytotoxicity of T-regulatory cells, whereas adhesion molecules of ICAM1 lymphocytes relate to a possible shortage of interaction between T-cells and MMSCs. The results open up new opportunities of analyzing MMSC samples, which can be of applied significance when these cells are used to ensure effective prevention and treatment of GVHD.

The value of intraosseous administration of MMSCs in patients with graft failure was studied. After the peripheral blood parameters were restored, bone marrow punctures were performed. Donor MMSCs were found in bone marrow aspirates 3 and 5 months after administration. In 1 patient, a small percentage of donor MMSCs were detected in 3 out of 4 samples, in another patient 3, donor cells were observed in one of 4 samples in 18.6%. During analysis of aggregate cellular production of MMSCs a tendency to a better growth of samples containing donor MMSCs was recorded in these patients. Thus, it was shown that functionally adequate donor MMSCs can remain in a patient's bone marrow for a long time.

3. 'Study of molecular, cytogenetic, and morphological basis of blood system diseases to detect molecular and biological markers, improve diagnostics, adequately select differential therapy and monitor the disease' (fundamental).

The most significant results obtained

Section: 'Study of hematopoietic precursors in patients with hemoblastoses after splenectomy'

As a result of the conducted studies, the characteristic dynamics of changes in both the absolute and relative number of hematopoietic progenitor cells in patients with hemoblastosis after splenectomy was revealed. In the majority of patients, a tendency to an increased absolute number of hematopoietic precursor cells are recorded following the conducted splenectomy during 7 days with a subsequent return to the initial value in 14 days. The obtained data are

extremely heterogenic for certain nosologies due to a small sample size. In a day following splenectomy, the relative number of stem cells in the peripheral blood tends to reduce.

Section: 'Role of oncogenic mutations in the prognosis of lymphoproliferative diseases'

Patients with diffuse large B-cell lymphoma (DLBCL) who underwent mNHL-BFM-90 or R-DA-EPOCH/R-HMA therapy were analyzed. One- and multivariate analysis was performed based on such criteria as gender, age, molecular type of DLBCL, nodal or extranodal type, presence of over 1 extranodal lesion, high activity of LDH, presence of B signs, stage and International Prognostic Index, high proliferative activity (Ki-67 > 80%), and gene MYD88 mutations. According to the analysis, the mutation status of MYD88 gene is an independent prognostic value when the treatment regimens are followed.

During the performed study, a method enabling to determine the presence of MYD88 mutations within formalin-fixed tissues in paraffin blocks and frozen DNA samples was developed. L265P MYD88 was found in 26.4% of nonGCB DLBCL. This corresponds to literature data.

Extranodal forms of DLBCL (78.5%) with high proliferative activity predominated in patients with L265P MYD88 mutations. Half of patients with DLBCL and MYD88-mut were over 60 years of age. This is most likely due to an increased number of nonGCB DLBCL in the older age group. Thus, in patients over 80 years of age, DLBCL of a postgerminal origin was diagnosed almost in 67% of cases as compared to 28% of cases among patients with DLBCL who are 50 to 60 years of age.

It is worth noting that L265P MYD88 mutation was found in 5 (55.6%) of 9 patients with DLBCL of immune privileged organs ($p = 0.04$).

A large number of works carried out over the past decade have revealed not only the cascade mechanisms of the functioning of the BKP, but also have led to the creation of targeted drugs lethal to tumor B cells. The use of medicinal agents such as BKP and TPR signaling pathway molecule inhibitors, which lead to the breakage of NF-kB activating signal, seems to be promising. It was established that the result of using a tyrosine kinase inhibitor, Ibrutinib, depends on the mutation level of BKP-NF-kB (CD79b and CARD11) signaling pathway major genes, and MYD88 gene mutation. In the study of patients with refractory/recurrent nonGCB DLBCL, ibrutinib was effective in patients with CD79b gene mutation. In case of combined CD79b and MYD88 mutations, a tumor proved to be more sensitive to the use of a targeted drug. Whereas the presence of the L265P MYD88 mutation and the wild-type CD79b gene is a predictor of resistance in patients with nonGCB DLBCL when using a BTK inhibitor. The molecular mechanism of the described interdependence of BKP and TPR signaling pathway gene mutations is unknown, as of today. Ibrutinib is also not effective in CARD11 gene mutations. Thus, ibrutinib should be used in patients with nonGCB diffuse large B-cell lymphoma and CARD11 wild-type gene, if no combination of L265P MYD88 mutation and wild-type CD79b is detected.

Two additional studies are planned to be carried out to identify mutations of CD79b and CARD11 genes in patients with nonGCB DLBCL. To stratify patients and determine the relevance of using target drugs, a mutation analysis of MYD88, CD79b and CARD11 genes is required. However, small-molecule monotherapy of patients with DLBCL is ineffective. As mentioned above, up to 80% DLBCL remissions were achieved owing to primary intense chemotherapy (CT). One of the probable reasons of tumor refractivity to even such supertoxic therapy are alterations of BKP and TPR signaling pathway genes, which require selective target drugs. Therefore, in our opinion, the prospect of using BKP signaling pathway molecule

inhibitors combined with intensive CT as an approach to the treatment of prognostically unfavorable variant of DLBCL deserves attention. Thus, new treatment options will potentially contribute to overcoming resistance mechanisms in patients with DLBCL who have proved resistant to intensive CT. The BKP and TPR signaling pathway molecular and genetic profile testing will ensure the accuracy of a therapeutic approach in patients with nonGCB DLBCL.

Section: 'Detection and monitoring of a PNG clone in patients with aplastic anemia and aplastic syndromes at different stages of the course of the disease'

The PNG-clone was determined using a standard method of highly sensitive flow cytometry as per the protocol suggested by the International group of flow cytometry researchers in 2010.

Based on the present test results, a PGN clone was newly found among primary patients with AA in 66% of cases. The rate of PNG clone detection in patients with severe and non-severe AA did not differ significantly and amounted to 62% and 71% respectively. However, it was shown reliably that an average clone size in patients with non-severe AA is higher and amounts to 19.48 ± 7.33% vs 3.63 ± 1.29% ($p = 0.043$). It is possible that a greater size of a PNG clone in patients with non-severe AA detected during a primary study is associated with higher heterogeneity of this group of patients with AA as compared with patients with severe AA. Although there is no difference in the duration of the disease from the occurrence of the first clinical symptoms to making diagnosis of AA and having a study to find a PGN clone, it can be suggested that a disease duration in non-severe AA is higher, whereas the depth of bone marrow aplasia is less than in case of severe AA. Favorable conditions are being created for the expansion of the PNG clone. Moreover, patients with non-severe AA more commonly go through a clonal evolution and development of PN, MDS and AML.

When analyzing the immunosuppressive therapy effectiveness, it was established that the majority of patients with AA and PNG clone required minimum therapy. The first course of ATG and cumulative response rate were significantly higher among patients with AA and PNG clone. 56% of patients with a PNG clone respond to therapy by month 6, whereas only 44% of patients with AA and without PNG display a primary response to treatment by the same time period. At the same time, the first course of ATG was followed by complete remission in the group with AA and PNG clone significantly more often (47% vs 17% respectively). Thus, the presence of a PNG clone in patients with AA found during the primary testing and prior to immunosuppressive therapy can be taken as a favorable prognosis of immunosuppressive therapy effectiveness, and factor of an earlier and more complete response to the performed therapy.

Based on the results of this study, the dynamic change in the size of the PNG clone, both in the direction of decrease, and in the direction of increase and appearance, occurs in response to IST, during remission, and most likely depends on the advantage to the growth of normal (GPI-positive) or clonal (GPI-negative) hematopoiesis.

In the course of the PNG clone dynamic testing, a variety of trends was recorded among patients with aplastic anemia during and after immunosuppressive therapy. At the same time, the analysis of data obtained during the follow-up period showed an increased number of patients with aplastic anemia and PNG clone of less than 10% (68% of patients before treatment and 82% of patients after treatment) with a simultaneous decrease in the number of patients with a minor PNG-clone.

In vitro experiments detected a reduced response to a cytotoxic effect of GPI-negative cells. GPI-negative hematopoiesis (PNG clone) is a shunting mechanism in case of self-aggression. Its proliferation, which is attributable to the external advantage, allows to adjust to the created

conditions. Significant changes in the size of a PNG clone during a response to IST (restoration of normal hematopoiesis when self-aggression is blocked) were observed. At the same time, over 50% of treatment response among patients with a PNG clone was achieved in case of a more intense exposure and during a larger period of time. GPI-defective cells appear in the peripheral blood of patients with AA without an initially detected PNG clone in response to therapy. They are very few. It should be noted that these patients responded to the minimum amount of IST. It seems that a more detailed assessment of bone marrow cell hematopoiesis and search for optimal laboratory approaches are necessary.

Section: 'Study of molecular mechanisms of pathogenesis and chemoresistance of aggressive and mature cell tumors of the lymph system based on the analysis of immunohistochemical and genetic mechanisms'

For the purpose of differential diagnosis of DLBCL with isolated mediastinal lesion and primary mediastinal large B-cell lymphoma (PMBCL), expression of JAK2, MAL, PDL1, PDL2 and TRAF1 genes were assessed. Expression of JAK2, MAL, PDL1, PDL2 and TRAF1 genes was seen in 17 (43%) of patients. PMBCL was diagnosed in 5 (30%) patients. 1 (20%) patient had an early relapse with CNS involvement.

Follicular lymphoma is a heterogeneous group of lymph tumors with various clinical manifestations, morphological variants and different responses to therapy.

In case of ALK+ anaplastic large cell lymphoma (ALCL) it was proven that the detection of chimeric oncogenes in the blood and/or bone marrow, formed as a result of translocations specific to this disease with the participation of the ALK gene, is a factor in an unfavorable prognosis. Minimal residual disease detected after treatment is an evident predictor of relapse or progressive disease. Thus, detection done with the method of online polymerase chain reaction was accompanied with monitoring of minimal residual disease in primary blood and/or bone marrow samples and at the next stage of treatment: following the 2nd and 6th course of chemotherapy, every 3 months from the moment of the end of treatment in the first 1.5 years of observation, and every six months for the next 3.5 years.

Mycosis fungoides (MF) accounts for more than 60% of primary cutaneous T-cell lymphomas. The prevalence is 6 per 1 million people. The disease course is progressive: there is gradual shift to the macular, plaque and tumoral stages. Extracutaneous generalization with affected lymph nodes and visceral organs is observed in over than 70% of cases. Lungs, spleen and liver are most commonly affected. It is very important to assess the extent of the process to determine the prognosis and select therapy. T-cell clonality can be detected during early stages by rearrangements of gamma T-cell receptor in blood and bone marrow aspirates. However, prognostic value of these phenomena is not determined. The rate of T-cell clonality was assessed; its prognostic value of detection by T-cell receptor gamma chain in the bone marrow and peripheral blood of patients with the early stages of fungal mycosis/Sezary syndrome was determined.

As a result of the study, protocols of diagnostics and treatment of lymphatic tumors were newly created and an algorithm of differential diagnosis of some aggressive lymphomas was developed. New diagnostic protocols, treatment regimens of lymphoproliferative diseases and prevention of therapy complications will promote a wider use of these methods in clinical practice, which can significantly improve survival of patients with lymphatic tumors.

Section: ' Examination of molecular mechanisms of bacterial and fungal resistance in patients with hemoblastoses'

Virulence genes of 363 hospital strains of *Enterococcus* spp isolated from hemoculture of patients with blood system tumors were studied. The isolated strains of *Enterococcus* spp. included 281 (77,4%) *E. faecium*, 74 (20,4%) *E. faecalis*, 8 (2,2%) others (5 *E. gallinarum*, 2 *E. durans* and 1 *E. hirae*). 37 (13.2%) strains of *E. faecium* and one (1.4%) strain of *E. faecalis* were resistant to vancomycin. Isolates of vancomycin-resistant *E. faecium* had the genes vanA (n = 29) and vanB (n = 8). The virulence genes were detected in 334 (92%) strains and absent in 29 (8%) of *Enterococcu*s spp. The esp (75,8%) and hyl (65,5%) genes were found significantly more often with *E. faecium*, whereas asa1 (62,2%) and gelE (68,9%) genes were discovered with *E. faecalis* ($p < 0.001$). While studying the virulence genes of *E. faecium*, which are resistant and sensitive to vancomycin, significant differences in the detection rate of one asa1 gene only were found. It was predominant among vancomycin resistant *E. faecium* (8.1% vs 1.6%, $p = 0.02$). Vancomycin resistant *E. faecium* with vanA genotype contained almost all studied genes, except for cylA, whereas strains with vanB genotype had only two of them (esp and hyl). It was determined that *E. faecium* is the dominant hemoculture-isolated *enterococcal spp*. The examined virulence genes were found in the majority of *Enterococcus spp* strains (92%). Various virulent genes are shown to dominate among various *enterococcal spp*.

Colonization of intestinal and nasopharyngeal mucous membrane with extended spectrum beta-lactamase (ESBL) products was studied in patients with acute myeloid leukemia (AML) and lymphomas prior to chemotherapy. Phenotypic methods and PCR method were used to detect ESBL and blaTEM/blaCTX-M resistant genes respectively. The prospective study (2013-2014) included 98 patients (33 patients with AML and 65 patients with lymphomas). 94 (96%) patients had newly diagnosed hemoblastosis. The median of age of patients with lymphomas and AML was 47 and 35 years respectively. Upon admission, colonization of intestinal mucous with ESBL producing enterobacteria was found in 26 (27%) patients (28% with lymphomas, 24% with AML and only 4 (4%) with oropharyngeal mucosa, $p < 0.01$. 34 isolates were isolated (E. coli 52%, K. pneumoniae 42%, Citrobacter spp. 6%). CTX-M type beta-lactamases were present in 76% of isolates, TEM type — in 53%, simultaneously two types — in 44%. Statistically significant colonization factors with ESBL producers included transfer to the National Medical Research Center for Hematology of the Ministry of Health of Russia from another hospital (OR 4.2; $p = 0.01$) and 50 years of age and older (OR 3.0; $p = 0.05$) for patients with lymphomas; living outside Moscow (OR 7.6; $p = 0.04$) for patients with AML. Similar factors were independent in multiple factor analysis. According to the study, almost every third patient (27%) with newly diagnosed AML and lymphomas admitted to the National Medical Research Center for Hematology of the Ministry of Health of Russia had colonized intestinal mucosa with enterobacteria and ESBL products. They were more frequently detected on the rectal mucosa as compared to the nasopharyngeal one. E. coli and K. pneumoniae, which mainly contained CTX-M beta-lactamases, prevailed among enterobacteria. The obtained results challenge administration of fluoroquinolones for prevention purposes without a preliminary examination.

The nature of infections and effectiveness of antibiotics were examined in patients with acute myeloid leukemia (AML) with/without colonization of intestinal mucosa with enterobacteria and ESBL products. A prospective study (2013-2015) included 66 patients with AML, who obtained 208 courses of chemotherapy during 6 months. Infectious complications were recorded in 193 (93%) chemotherapy courses. The analysis included 173 episodes of infections; 68 and 105 of them were with and without colonization of intestinal mucosa with enterobacteria and ESBL products. Indications to administration of antibiotics were comparable in patients with and without colonization with ESBL products, except for the cases of bacteremia due to ESBL-producing Enterobacteriaceae, seen in patients with colonization only (7.5%; $p = 0.009$). Patients with and without colonization with ESBL producers had comparable outcomes regarding effectiveness of antibiotics during 1 stage of therapy (38% vs 44%), antibiotics substituted by carbapenems (62% vs 55%), effectiveness of monotherapy (36% vs 52%) and combined therapy

(64 vs 41%) with carbapenems, duration of aggregate use of antibiotics (14 vs 13 days) and separate use of carbapenems (10 days each). With carbapenem, all ESBL-producing bacteremia cases were treated.

It was shown that colonization of intestinal mucosa with ESBL-producing Enterobacteriaceae is predictor of bacteremia caused by similar microorganisms. No differences were found in the use of antibiotics among patients with and without colonization of the intestinal mucosa with ESBL producers.

Section: 'Study of molecular, biological and instrumental research methods in patients with multiple myeloma at different stages of high-dose chemotherapy'

The genetic structure of multiple myeloma (MM) has primary chromosomal abnormalities represented by t(14q32)/IgH with loci of the genes regulating the D-type cyclins and multiple trisomies with an increased number of copies of potential oncogenes. Secondary abnormalities associated with tumor transformation include del13q14, amp1q21, del17p13/TP53, and t(8q24)/cMYC. From 07.2009 to 09.2016, 134 patients (67 men and 67 women) aged 27-81 (median 57) years underwent FISH-analysis of mononuclear cells and CD138+-cells of bone marrow prior to treatment to find tIgH, and trisomy 5, 9, 15. Induction therapy included 6-8 bortozemib-containing courses. 48 patients had autologous stem cell transplantation. The follow-up period for patients was 19.3 months. tIgH, trisomies and hypoploidy were found in 57 (42.5%), 77 (57.5%) and 3 (2.4%) of patients respectively. The first two were combined in 11.2% of cases. The incidence of separate t(14q32) was as follows: 16,4% for t(11;14); 12,7% for t(4;14); 3,7% for t(14;16) ; 2,2% for t(14;20); t(6;14) for one patient; in 6.7% of cases no chromosomal partner was established. Del13q14 was found in 54 (40,3%); delTP53 in 17 (12,7%); t(8q24) in 23 (17,2%); and amp1q21 in y 53(39,6%) patients. Chemosensitivity of MM was assessed in 127 patients. The best response was found in patients with t(4;14) (frequency of CR 18.8%), hyperdiploidy (frequency of CR 17.8%), t(11;14) (frequency of CR 14,3%), del13q14 (frequency of CR 13,7%) and amp1q21 (frequency of CR 11,8%). Tumor sensitivity to bortezomib-containing programs was significantly worse in patients with delTP53 (frequency CR 5,9%) and t(8q24/cMYC). No complete remission was achieved in this group of patients. The obtained results were processed to assess the effect of chromosomal aberrations on the long-term tumor prognosis. Statistically significant differences in total survival (TS) were obtained within groups of patients with delTP53 ($p = 0,02$), amp1q21 ($p = 0,07$) and t(8q24/cMYC) ($p = 0,001$). In addition, it was found that 3 copies of 1q21 produced no significant effect on disease prognosis. Patients with over 3 copies of 1q21 had statistically lower values of total survival as compared to groups without amp1q21 and three copies of 1q21. Deep analysis of the obtained results has shown that 17p13/p53, amp1q21 and t(8q24/cMYC) deletion are factors of unfavorable prognosis in patients with MM.

In the prognosis of MM, the incidence of bone and extramedullary plasmacytomas is 7-18% with relapse occuring in more than 20% patients. The clinical course of MM complicated with plasmacytomas has poor prognosis, resistance to therapy, and low overall survival. An IHC test was done using tumor trepanobioptate and bioptate paraffin blocks to study molecular and biological features of tumor cells in the bone marrow and plasmocytomas in primary patients with MM. CD56, Ki67, CXCR4, and CD 166 antibodies were used (28 patients). 19 patients underwent ICH testing with trepanobiptate paraffin blocks and c-myc/ cyclinD1 antibodies. During an ICH trepanobioptate test it was found out that patients with MM and without plasmacytoma had CD56 expression (80% vs 38.5%, $p = 0.05$) and high level of Ki-67 expression (53.3 % vs 0%, $p = 0.002$) as compared to those with plasmacytoma. All patients without plasmacytoma had CXCR4 expression with tumor substrate cells in the bone marrow. This significantly increased the same value in patients with MM and plasmacytomas (100% vs 46.2%, $p = 0.001$). Analysis of ICH results and their comparison with the tumor process showed

that lack of CXCR4 expression in the bone marrow can be associated with a more aggressive course of the disease. Hyperexpression of c-myc protein in the bone marrow of patients with MM is an unfavorable prognostic marker regarding a deep anti-tumor response to bortezomib-containing therapy.

To determine the significant effect of mutation in exons 4 and 5 of UMOD on cast nephropathy (CN), UMOD was performed for genomic DNA from the peripheral blood mononuclear cells of 20 patients with MM and urinary excretion of Bence-Jones protein of more than 2 g per day. The basic group included 13 patients with CN. The comparison group consisted of 7 patients with MM and normal kidney function. 6 types of mutations were discovered: 4600 C> CT 277 C> C/C; 4870 G> GA 367 V> V/V; 4503 G> GA 245 R> R/G; 4918 C> CT 383 P> P/P; 5068 G> GA 398 G> G/G; 6979 G> GA 398 G> GA (NCBI Reference Sequence: NT_010393). Only one mutation (4503 G>GA 245 R>R/G) in a patient with CN results in amino acid substitution. No related information is present in the database. Other mutations are widely used polymorphisms (to 40% in the European population). No significant data about their effect on the course of any diseases are provided. These mutations were found among patients of both genders with an equal frequency. Thus, an association between UMOD mutations and CN was not established in MM. Polymorphisms in the UMOD gene were equally common in both subgroups, which indicates the absence of their influence on the development of CN.

Section: 'JAK2, MPL, CALR, FIP1L1-PDGFRa, CSF3R, TET2, SFRS2, SF3B1 gene mutations in myelodysplastic, myeloproliferative (MDS/MPD) and Ph-negative neoplasms: association with clinical features and pathomorphology'

Objective: to study molecular mechanisms of pathogenesis of Ph-negative chronic myeloproliferative diseases and to develop and introduce into clinical practice diagnostic and molecular-directed therapeutic approaches for Ph-negative myeloproliferative diseases.

Morphological features and histotopography of myelopoiesis fragments in bone marrow trepanobioptates were analyzed using the histological treatment method in patients with various nosological forms of Ph-negative MPD (true polycytemia, essential thrombocythemia, primary myelofibrosis and non-classified MPD). The degree of reticulin fibrosis was assessed using the histochemical method of Gomori staining in patients with Ph-negative MPD. It was concluded that a histochemical test of bone marrow trepanobioptates makes it possible to verify the histological stage of the disease and diagnose the disease at early stages.

A highly sensitive and highly specific molecular diagnostic method for diagnosing mutations of the *JAK2*, *MPL, CALR, SRSF2, SF3B1 and ASXL1* genes was developed. The testing was done using the retrospective paraffin and frozen samples (tissues, cells and nucleic acids) stored in the database of laboratories for the studied nosologies, as well as prospectively on blood samples of patients.

It was established that in cases of hidden MPD and having only venous thrombosis of the portal system as clinical manifestations, the detection of the JAK2V617F mutation and subsequent histological examination of the bone marrow can confirm the diagnosis of MPD. Timely cytoreductive therapy, as well as anticoagulant therapy, make it possible to achieve recanalization, but an individual approach and an assessment of the risk of bleeding are required.

However, monitoring results of hematological and histological responses and qualitative assessment of molecular responses (JAK2V617F) in patients with Ph-negative MPD against the background of interferon therapy displayed the possibility of hematological, molecular and histological response in patients with TP and ET.

Section: 'Mutations of signaling pathway genes and lymphocyte receptors: the role in pathogenesis and therapy sensitivity in lymphoproliferative diseases'

40 patients with hairy cell leukemia (HCL) and 24 patients with splenic marginal zone B-cell lymphoma (SMBCL) were examined to detect *BRAF V600E mutations.* The mutation was found in 39 of 40 cases of HCL and in none of the cases of SMBCL. Taking into account that pathogenic mutations in the *BRAF* gene are not limited to the *V600E* variant, and that the main number of activating mutations is localized in exons 11 and 15, we studied the nucleotide sequences of the corresponding exons of this gene in all patients included in the study. No activating mutations were found at exons 11 and 15 of the *BRAF* gene in any of the patients with HCL or SMBCL.

According to the literature data, in the variant form of HCL and in cases when *IGHV4-34* is expressed during HCL, activating mutations in the *MAP2K1* gene encoding the MEK1 MAP kinase are detected. To assess the frequency of these mutations, we have developed a diagnostic system based on Sanger sequencing. C.167A>C mutation resulting in the substitution of Q56P in the aminoacid consequence of MEK1 protein was found in 1 patient with HCL. While determining the mutational status of immunoglobulin genes, it was found out that this is the only patient within our sample related to the subgroup of *IGHV4-34* positive HCL. It is important to note that this case had an atypical picture of bone marrow damage — morphologically only scattered lymphoid cells were detected, but immunohistochemical examination revealed pronounced interstitial lymphoid infiltration by CD20+CD79a+ B cells. At the same time, in the biopsy of the removed spleen, morphologically, immunohistochemically and phenotypically, the diagnosis of HCL was confirmed, while a repeat study showed the presence of a mutation of *MAR2K1 Q56P* and the absence of a mutation of *BRAF V600E* in the spleen tissue. None of the 24 patients with SMBCL had mutations in the *MAR2K1* gene.

A number of molecular and genetic studies were conducted in 5 patients with a splenic cord lymphoma. No *BRAF V600E* mutations were found in the samples. Sequencing of exons 11 and 15 of the BRAF gene and 2, 3 and 11 exons of the *MAP2K1* gene demonstrated the presence of G128D activating mutation of the *MAP2K1* gene in one of the patients. Four patients had mutated genes in the variable region of immunoglobulins (IgVH). VH genes of the 3 and 4 families were used in rearrangements. No mutational status could be established in one patient.

Section: 'Study of the genetic structure of leucosis ad lymphomas: determination of basic molecular and cytogenetic events in the basis of initiation and progression of tumors'

Complex changes in the karyotype of patients with MDS and AML are factors of unfavorable prognosis. They can be the result of a multiple stage process with a gradual accumulation of chromosomal abnormalities, i.e. evolution of a tumor clone. Possibilities of a standard cytogenetic testing (SCT) are limited due to a low resolution of this method. Molecular and cytogenetic methods such as interphase fluorescence in situ hybridization (iFISH), multicolored fluorescent in situ hybridization (mFISH), and multicolored identification of high resolution chromosomes (mBAND) make it possible to identify complex chromosomal disturbances, marker chromosomes, submicroscopic deletions and translocations with loci deletions of known and potential genes participating in the disease pathogenesis. At the time of diagnosis, these methods were used in 438 patients with MDS and 164 patients with AML. The present study included 27 patients with complex karyotype disturbances (13 men and 14 women, average age 59.8 years): 15 patients with MDS (3.4%) and 12 patients with AML (7.3%). These patients underwent iFish, mFISH, and in three cases mBAND was performed for chromosomes 5, 7, 15, 17. In the result of a standard cytogenetic assay (SCA), 7 abnormalities per karyocyte (3-21) were found in complex karyocytes. Additional unidentifiable chromosomal material was detected in 18 cases (66.6%), marker chromosomes — in 13 cases (48.1%). Additional

chromosomes (tri- and tetrasomy) were detected in 8 cases, monosomies were much more common, in 19 cases. Chromosomes 5 (40,7%), 7 (18,5%) and 17(11%) were most often absent. As a result of the study, molecular cytogenetic methods revealed an average of 6 anomalies per karyotype (from 2 to 16), Chromosomal disorders are mainly represented by translocations (72%), 51 simple reciprocal translocations, 21 translocations involving three or more chromosomal partners were identified. True monosomy 7 was found in one patient and no true monosomies were found for chromosomes 5 and 17. Marker chromosomes and additional non-identified chromosomal material, except for one case, were identified as chromosomal material occurring due to imbalanced translocations. In the result of SCA, monosomy 7 was detected outside the complex karyotypes as well. In patients with MDS, monosomy 7 was found in 2.5% of cases as the only chromosomal disturbance combined with any disturbance in less than 1% of cases. Monosomy 7 was found in simple karyotypes of patients with AML in 7.2% of cases. In 4.2% of cases it was the only chromosomal disturbance. Fragments of chromosome 7 were not detected with molecular and genetic methods. A combination of mFISH, iFISH and mBAND methods allowed to identify all non-SCA identified qualitative and structural abnormalities, determine occult chromosomal disturbances, specify chromosomal breakpoints and determine the origin of marker chromosomes. In complex karyotypes, true monosomies 5 and 17 were not detected in any case; true monosomy 7 was found only in one patient. In addition to this case, molecular cytogenetic research methods revealed fragments of chromosomes 5, 7 and 17 involved in simple reciprocal and complex translocations, which, except for one case, were accompanied by deletions of regions 5q31, 7q31 and 17p13. Monosomy 7 found in simple karyotypes of patients with MDS and AML is always true (fragments of chromosome 7 were not detected).

Disorders of the hereditary apparatus have an important place in human pathology. A significant part of ontogenesis disorders is associated with numerical or structural aberrations of the entire genome or individual chromosomes. Genetically balanced chromosomal aberrations (translocations, insertions, inversions), as a rule, do not affect the phenotype of the carrier. Chromosomal mosaicism in chromosomal diseases has been described for sex chromosomes and many autosomes. An increased risk of leukemia in some hereditary syndromes has been proven: Down syndrome, Fanconi anemia. It can be assumed that constitutional rearrangements lead to the appearance of chromosomal instability of the region; genes localized at the points of chromosomal breakpoints lose their usual orientation and, probably, can change their functional activity. Information about the frequency of oncohematological diseases in individuals with constitutional rearrangements of chromosomes is not widely reflected in the literature. Constitutional structural aberrations with checkpoints, which look like leukemia-associated checkpoints, were recorded in patients with onchematological diseases. It remains unclear whether constitutional aberrations play a significant role in leukemogenesis, or whether the development of malignant blood diseases in patients with constitutional karyotype changes is random. A retrospective study included an absolute number of constitutional rearrangements in patients from the National Medical Research Center for Hematology based on the results of a standard cytogenetic testing in 2014-2016. 3,503 patients were included into the study. Cytogenetic findings were detected in 54 patients (32 women and 22 men) aged 19-81 with various hematological diseases who underwent standard cytogenetic testing (of bone marrow in 53 cases and splenic bioptate in 1 case); the constitutional nature of rearrangements in cytogenetic analysis of PHA-stimulated peripheral blood T-cells was also confirmed in 12 cases. Two studies with in situ hybridization (FISH) and DNA probe (CEP X SpectrumOrange/Y SpectrumGreen DNA Probe (ABBOTT)) were performed in patients with sex chromosome abnormalities. During the presented period, six translocations (t) were detected: 4 reciprocal translocations (women) and 2 Robertsonian translocations (1 in a woman and 1 in a man). Two pericentric inversions, additional supernumerary marker, and numerical abnormalities of sex chromosomes were detected. Normal variants of chromosomal polymorphism were analyzed.

The number of identified structural and numerical anomalies, the frequency of which is known in the population, is significantly lower than the population value. Groups of patients who needed to undergo a PHA-stimulated peripheral blood lymphocytes study to exclude or confirm the hereditary nature of chromosomal aberrations were identified.

One of the most important factors determining the prognosis of the clinical course of CLL is chromosomal abnormalities. The use of FISH using probes to loci characterized by aberrations makes it possible to detect chromosomal abnormalities in 80% of CLL patients. However, karyotype changes in CLL patients are not limited to these chromosomal disorders. It has been shown that the presence of any translocations has an adverse effect on the course of CLL. Moreover, recent studies report that the complex karyotype significantly worsens the results of treatment with ibrutinib in patients with resistant and recurrent CLL compared with the deletion of 17p13/p53, determined only by the FISH study. The aim of this work was to characterize the karyotype of immuno-stimulated peripheral blood B-lymphocytes in patients with chronic lymphocytic leukemia. Cultivation was carried out with the addition of an oligonucleotide division stimulator DSP 30 and interleukin-2 and using a standard combination of LPS and TPA mitogens. 34 patients with B-cell chronic lymphocytic leukemia who were observed from November 2015 to November 2016 who underwent standard cytogenetic testing (SCT) and in situ fluorescent hybrdization (FISH) were analyzed at the National Medical Research Center for Hematology of the Ministry of Health of Russia with 18 men and 13 women (men and women ratio is 1.4) being 38-86 years of age (average 60 years of age, age-related median is 58 years). Chromosomal aberrations were recorded in 28 patients (82%): 1, two and three aberrations were found in 8 (23%), 9 (27%) and 11 (32%) patients. Translocation t(11;14) was detected in 3 patients, in 2 of them it was combined with a complex karyotype. During karyotyping, the dividing cells were found in 31 patients (91%). A normal karyotype was found in 3 (8%) of patients. However, 1 (3%) of them had aberrations found during a FISH testing. Complex karyotypic disturbances were found in 11 (32%) patients; a deletion of the short arm for chromosome 17 was found during FISH-testing in 5 (15%) patients. Such a high percentage of complex karyotype detection compared to the data of foreign authors (16%)it can be explained by analyzing a large number of metaphases (from 40-80) and taking into account all clonal disorders, including the deletion of the Y chromosome.

Section: 'Study of molecular, genetic and cytogenetic traits in chronic myeloid leukemia and myeloproliferative disorders with eosinophilia and identification of novel molecular and biological predictors of Ph-positive tumor clone reduction to optimize target therapy with inhibitor of tyrosine kinases'

Evolution of a leukemic clone was analyzed under the effect of target therapy with tyrosine-kinase inhibitors, including assessment of the value of cytogenetic, molecular and genetic methods of characteristics of the tumor clone volume in case of CML, assessment of possible elimination of a leukemic clone in myeloproliferative disorders with eosinophilia, identification of CYP gene polymorphism as predictors of tumor clone reduction in case of CML, pharmacokinetic modelling of TKI transplacental passage and comparison of these data with *in vivo* results. The data can form the basis of optimized target therapy in CML and MPD with eosinophilia.

When the outcomes of a standard cytogenetic testing and MT as methods estimating the volume of a leukemic clone in case of CML were compared, their positive correlation was confirmed (correlation coefficient 0.715). It was established that in the lack of PCR and at the level of Ph-positive cells within the range of 100-36%, the level of *BCR-ABL* is varied significantly and cannot be the only marker to assess the tumor clearance at early stages of TKI. It is noted that at a BCR-ABL level of less than 1%, 86% is characterized by the presence of PCR, and this level

can be considered as an equivalent of PCR in the absence of cytogenetic monitoring. At a BCR-ABL level of more than 10%, the absence of CR or minCR is mainly detected.

When studying the dynamics of PDFRA, PDGFRA clones and the possibility of their complete elimination under the influence of TKI in MPD occurring with eosinophilia, it was found that targeted therapy with imatinib allows obtaining CHR in 90% of cases within 1-3 months. MO can be achieved in 88% of cases with a follow-up of 7 months. In the future, the loss of MR is possible; further observation is required to generalize data on the dynamics of the leukemic clone in MPD with eosinophilia.

When analyzing polymorphisms of CYP and methyltransferase genes as predictors of tumor clone reduction in CML, it was found that in the group with resistance to TKI, the wild-type genotype was 4-9 times more common than in the group with high sensitivity to TKI, the sensitivity of the test was 50%, specificity 97%, positive prognostic value (PPV) and negative prognostic value (NPV) of 89% and 79%, respectively. Subsequent prospective data collection is required to assess effectiveness of the test.

In pharmacokinetic modeling of TKI transplacental transfer and comparison of these data with *in vivo* results it was established that all TKIs used in CML can passively penetrate through the BPB based on physical and chemical properties. The ratio of imatinib and nilotinib in the plasma of a mother and a fetus was significantly lower than the target value and amounted to 0.05-0.22 (Me 0.12) for imatinib and 0.5-0.58 for nilotinib. Thus, a decreased risk of the TKI exposure due to limited penetration through the BPB was confirmed. The difference in theoretical evaluations and practical data is most likely explained by an active transport of medicinal drugs and individual traits not taken into account during theoretical modeling.

4. Determination of clinically relevant minor histocompatibility antigens in HLA-similar transplantation of hematopoietic stem cells (fundamental).

The most significant results obtained

The study of the properties of minor histocompatibility antigens (MHA) and the discovery of new ones is an important task. The first MHAs were discovered in the 1990s, and over the past two decades, with the development of technologies and the emergence of new approaches, such as genome-wide association search (GWAS, Genome-Wide Association Studies), researchers find new minor antigens annually. We have analyzed data from about 50 different MHAs. The accumulated knowledge on this topic will help to individually approach each patient undergoing the procedure of allogeneic hematopoietic stem cell transplantation (HSCT). For this, prior to surgery it is necessary to obtain data about non-correspondence of donor and recipient genotypes by known MHAs. In addition to HLA typing, MHA genotyping is a test, which has to be performed prior to HSCT. It helps to select an optimal donor, predict an intensity of an allogenic immune response (graft-versus-host disease), modify a graft to prevent adverse reactions and intensify a cytogenetic graft effect in relation to residual tumor cells. In spite of the obvious importance, MHA genotyping prior to HSCT is not performed for clinical purposes. Moreover, no approach for fast and reliable typing of patients by MHA selected panels has been developed based on a human HLA genotype. The methods described in the literature for determining allelic variants of MHAs are suitable only for the analysis of individual polymorphisms and are unsuitable for working with a large volume of clinical material and parallel analysis for several parameters. The only currently available approach for one-time genotyping of MHAs includes 10 antigens, and due to the peculiarities of the method, it is not possible to expand this panel. To develop an optimal approach, we analyzed four methods used for MHA typing: allele-specific PCR (AS-PCR), melting curve analysis method, quantitative PCR using TaqMan probes and the

Restriction Fragment Length Polymorphisms (RFLP) method. Sanger sequencing was used as a control method. The parameters for evaluating the methods were simplicity of execution, time spent, cost, the possibility of compiling individual panels for patients with different HLA genotypes. Quantitative PCR using fluorescent probes turned out to be the most suitable. We modified this approach using allele-specific primers, which allowed us to maintain high accuracy and reliability with high productivity of the method. In addition, this approach works on a modular principle, in which MHAs are combined into panels based on their restrictive HLA allele. Thus, only the required number of panels is used for each patient. The use of probes with different fluorescent labels reduces the number of reactions. Currently, the panel for genotyping MHAs restricted by the HLA-A*02 allele, which is carried by more than 30% of the inhabitants of the Russian Federation, is fully ready and worked out. The panel includes all known 16 minor antigens (for HLA-A*02), and its expansion is possible as new ones are discovered. In addition, panels of 16 minor antigens covering 9 more HLA alleles are under development.

Using the developed approaches, an active study of the genetic material of patients and their donors who were previously treated at the Hematology Research Center, or are currently being treated, is being conducted. To date, 27 donor-recipient pairs have already been partially analyzed, and information on them is being updated as typing capabilities expand.

To study the role of individual antigens in the in vitro system, the potential detection of new histocompatibility antigens, a collection of lymphoblastoid cell lines (LCL) of patients is being created, as well as a cryo-bank of peripheral mononuclear venous blood donors. The collection currently includes 16 LCL lines and is expanding as new patients arrive.

5. Changes in the stromal microenvironment of the bone marrow under the influence of cytotoxic drugs and tumor cells during the treatment of patients with hemoblastosis (fundamental)

The most significant results obtained

The subject of the study is stromal hemopoietic microenvironment precursor cells such as multipotent mesenchymal stromal cells (MSC) and fibroblast colony-forming units (FCFU) in the bone marrow of patients with hemoblastosis.

The paper shows that the concentration of FCFU is significantly and statistically decreased at the time of diagnosis of the disease in the bone marrow of patients with AML and ALL. Decrease in fibroblast colony-forming units in patients with AML and ALL at the disease onset did not correlate with the number of blasts in the bone marrow. Thus, the observed decrease in the number of stromal precursors cannot be explained by the fact that stromal cells were replaced with blasts in the studied samples. Concentration of fibroblast colony-forming units (CFU) was only partially restored in the bone marrow of patients with AML, whereas in patients with ALL, the level of CFU was restored already in 37 days after treatment initiation and did not decrease further.

The total cellular production of MSCs in patients with all the nosologies studied did not significantly differ from that in the cultures of healthy donors. The time required for the formation of a confluent monolayer after the initial bone marrow planting (time up to P0) was increased in all nosologies studied (AML, ALL, CML). Since the time between the next passages of donor and patient cultures did not differ, it can be assumed that the number of MSCs capable of proliferating and initiating the formation of a confluent monolayer was reduced in the initial bone marrow samples of patients.

At the same time, an ability to support the growth of hematopoietic precursors (Cobblestone Area Forming Cells, on day 7 of cultivation) from the bone marrow of healthy donors was changed in the mesenchymal stem cells (MSCs) of patients with different nosologies. At the time of diagnosis, the MSCs of patients with AML and ALL were supported by early hematopoietic precursors in a significantly and statistically worse way as compared with MSCs of healthy donors; MSCs of patients with CML displayed improvement of this ability compared to the control. A significantly changed expression of various growth factors, differentiation markers and other regulatory molecules were found during analysis of the relative level of gene expression in MSCs of patients before treatment. It is obvious that development of the studied leukemia changes stromal precursors in the bone marrow of patients.