SUMMARY OF FINAL ANALYTICAL REPORT

Topic 1. Study of molecular biological and biophysical mechanisms of pathological processes in diseases of the blood system

Mutation analysis systems have been developed and a search for pathological variants of von Willebrand genes, factors V, VII, VIII, XI and XII has been carried out. Mutations with different types of Willebrand's disease have been identified. A total of 11 different gene defects have been identified. Two missense mutations were new (Pro2527His and Ala2178Ser). The most common was microdeletion c.2430delC, most often found in patients with type 3 disease. It was shown that the high rate of His634Arg mutation in the Sverdlovsk region, which is responsible for mild hemophilia A, is due to the founder effect. Mutations in the F8 gene were identified in 30 patients with inhibitory hemophilia A. The majority of patients had severe mutations (inversions, nonsense mutations, frameshift mutations, and massive deletions). Missense mutations were found in 3 patients only. The distribution corresponds to the idea of correlation between the severity of a genetic defect and development of the inhibitory disease. Both new, and major mutations in the F7 and F12 were detected in patients with hypoconvertinemia and Hageman disease. The systems of full-scale mutational analysis for V and XI factor genes were developed and successfully approved. New mutations in the F5 (Cys684Tyr) and F11 (c.1767 delG) genes were found.

The Rh phenotype was found in 3,269 patients. Antigens C and D were identified in 46 patients who underwent genotyping. Genotyping revealed the presence of the rare RHCE*Ce09.01 gene in two individuals and confirmed the presence of the gene. Two people with DVII and DNB partial antigens confirmed at the molecular level were found. Dweak type 67 (1) and Dweak type G763A(1) antigens identified with sequencing were identified and characterized for the first time. Genotyping was performed on 36 individuals with weakened antigen D. 11 of them had Dweak type 1, 5 had Dweak type 2, 19 had Dweak type 3, and 1 had Dweak type 15. To resolve the ambiguities of genotyping by allele-specific PCR, highly specific PCR systems for differential testing of RHD and RHCE genotypes have been developed and successfully tested. During the reporting period, PLT aggregation was recorded under high shearing stress at a flow meter. The number and sizes of aggregates were analyzed with micrography and Python scripts (ready-made tools). In series of experiments it was established that an increased shift rate results in a decreased number of aggregates when their size is increased up to 100 micrometers.

A mathematical model describing the hydrodynamic activation of PLT during the transitional mode of flows was improved.

Topic 2. Study of immunogenicity of variant peptides encoded by human genomic polymorphisms

A method of rapid genotyping of the selected panel of polymorphisms was developed based on real time PCR using fluorescent probes.

Control DNA matrices coding all allele variants for every polymorphism were created.

Topic 3. Study of molecular, cytogenetic, immunomorphological bases of the pathogenesis of clonal diseases of the blood system

A lack of association with chemotherapy program was found during the study of MUM1 expression in a follicular lymphoma. Irrespective of therapy choice, 5-year overall survival rate was 100%. It can partially be explained by a good anti-tumor T-cell response, as evidenced by parafollicular location of T cells, and a well-defined network of follicular dendritic cells.

Highly sensitive qualitative methods determining RHOA G17V; STAT3 Y640F; STAT3 D661Y mutations were developed. Specificity and sensitivity of the research of these mutations were determined in angioimmunoblastic T-cell lymphoma (AITL) and T-cell large granular lymphocyte leukemia (TCLGLL). A large control group of patients with various hematological disorders was examined to test specificity of the methods. The method of LNA-modified primers was newly used to assess these mutations. This increased the sensitivity of tumor cell detection to 0.02% and was sufficient to monitor the minimal residual disease (MRD) and estimate the amount of tumor cells in different tissues.

Significant differences in the IGHV gene repertoire were found for CLL, HCL, and splenic marginal zone lymphoma (SMZL). There was a different incidence in families and separate genes of immunoglobulin. There was a trend to stereotypy of antigenic B-cell receptors in SMZL and MZL. Potential SAR in SMZL and MZL differ from those in CLL.

In progressive B-cell chronic lymphatic leukemia, the percentage of CD80 and CD86 on tumor cells is lower than in primary patients with CLL. In patients with stage C, the percentage of B-cells with antigen expression was lower than in patients with stages A and B. It was established during our studies that the percentage of CD95-CD28+ T-helpers is lower in patients with disease progression as compared to donors and primary patients. The percentage of cytotoxic T-cells was lower for groups 1 and 2 as compared to donors. Meanwhile, the percentage of CD95-CD28+ cytotoxic T-cells was lower at the stage of progression as compared to primary patients. A decreased pool of CD95-CD28+ T- cells in patients with progressive CML could be associated with the effect of previous therapy or inhibitory action of tumor cells in CLL. We also established that in patients with progressive CLL, T-cells are characterized by a more significant expression

of PD-1 as compared to primary patients. It was shown that in CLL, T-cells (CD4+ and CD8+) have the so-called exhaustive phenotype (CD4+PD-1+ and CD8+PD-1+), i.e. PD-1 hyperexpression, with their secretory function being disturbed as well. The data obtained can indicate a constant antigenic effect of tumor cells on T-cells, CD95– CD28+ T-cell pool exhaustion, increased number of memory T-cells and T-effector cells, and an increased share of T-cells with PD-1 co-expression.

Multiple myeloma (MM) is characterized by a pronounced genomic heterogeneity due to multiple numeric and structural chromosomal changes. The changes are essential in oncogenesis and result in gene imbalance, changed gene structure and function, and, as a consequence, disturbed regulation of cellular cycle and cellular differentiation. FISH-analysis of mononuclears and bone marrow CD138+cells was performed to detect tIgH: t(11;14), t(4;14), t(14;16), t(14;20), t(6;14), del13q14/-13, del17p13/TP53, amp(1q21), del1p32, t(8q24)/cMYC, and trisomy 5,9,15. During the study, 35.7% of patients had t(14q32)/IGH, 44% of patients had trisomy; in 9.7% of cases they were combined, whereas in 10.6% of cases the primary chromosomal disturbances were not detected. The incidence of separate t(14q32) was as follows: 21.3% for t(11;14); 13.9% for t(4;14); 4.2% for t(14;16) ; 1.3% for t(14;20); 0.9% for t(6;14); in 4.2% of cases no chromosomal partner was established. Del13q14/-13 was detected in 45,4%; amp1q21 in 38,4%; t(8q24) in 13,4%; del17p13/TP53 in 12%; del1p32 in 5,1%; amp(8q24)/cMYC in 3,2%. In 20 patients of 22 (90.9%), no changes in the cytogenetic picture of the progressive disease were detected during the FISH study; 2 patients of 22 (9.1%) had amp1q21. Due a limited number of patients who underwent the FISH-analysis both at myeloma onset, and relapse/progression, it can't be asserted that tumor progresses because of the aberration only. In addition, this aberration was detected in 45.5% of patients already at the onset of the disease, and it is also known that the frequency of detection of amp1q21 increases in progression. Performing a FISH study both at the onset of the disease and in progression is important for clinical practice, since the identification of new chromosomal disorders can be a justification for timely changes in the tactics of treatment of MM patients.

The probability of maintaining the MRD-negative status after transplantation of autologous blood stem cells was determined It amounted to 60% for 3.5 years. The number of residual aberrant plasma cells in the bone marrow on the 100th day after auto-transplantation significantly affected survival rates, so the median PFS in the presence of more than 0.05% was 8 months versus 23 months when less than 0.05% of aberrant plasma cells were detected.

In a group of refractory patients with aplastic anemia, the percentage of NKT-cells, CD4+ memory cells, and activated (CD25+) CD4+ cells was significantly higher than in healthy donors ($p < 0.024$). The percentage of naive PD-L1-producing CD4+, CD4+ and CD8+ cells was lower

than in the control group ($p < 0.0187$). In primary patients, the percentage of effector CD4+ and CD8+ cells and CD4+ memory cells was higher ($p < 0.04$), whereas the number of naive CD4+, CD8+ and CD4+PD-L1+ cells was lower ($p < 0.02$). In the group of primary patients, a number of NKT cells was lower than among refractory patients ($p = 0.03$). In the remission group, the number of CD4+ naive cells and regulatory T-cells was lower than in the control group ($p <0.048$), with the proportion of CD4+ effector cells and memory cells being higher ($p < 0.025$). As compared with refractory patients, patients in remission had more CD4+PD-L1+cells and less CD4+CD25+ cells.

While analyzing the evolution of changes in the percentage of various subpopulations of T cells, it was found that a relative number of NKT-cells, CD3+CD4-CD8-cells and activated T cells in primary patients was increased during therapy. It was also discovered that a relative number of regulatory T cells and effector CD4+ cells dropped during therapy.

Expansion of at least one Vß subclone among CD8+ cells was detected in all primary AA patients. In patients in remission, subclone expansion was not always detected among T cells, which indicates the effect of therapy on the subpopulation composition of T cells.

50 (15%) of vancomycin- resistant Enterococcus (VRE) were identified, of which 33 (66%) and 17 (34%) had the vanA and vanB genes of resistance to glycopeptides respectively. Linezolid-resistant E. faecium isolate was found in 2012 (MIC 16 mcg/ml). During sequencing of linezolid-resistant E. faecium, mutations in the 23S rRNA coding gene were detected. One E. faecalis isolate with moderate resistance to vancomycin and vanD, and one vancomycin-resistant E. gallinarum isolate with vanC1 and vanB were detected during the study.

Formation of biofilms was examined in 109 Candida spp. (C. albicans n = 22, C. parapsilosis n = 22, C. tropicalis n = 22, C. krusei n = 21, C. glabrata n = 22) isolated from hemoculture. Biofilms were formed in 54% (n = 59) of Candida spp. The rate of biofilm formation was higher among Candida non-albicans (60%) as compared to C. albicans (32%, $p = 0.02$). C. tropicalis (82%) and C. krusei (81%) isolates formed biofilms more frequently than C. parapsilosis (50%), C. albicans (32%) and C. glabrata (27%, $p > 0.05$). Distribution of the blaCTX-M, blaTEM and blaSHV genes were examined in 499 Enterobacterales isolates producing extended spectrum beta lactamases. The blaCTX-M genes were most common (82.6%). They were followed by the blaTEM (73,7%) and blaSHV (53,7%) genes. The studied blaCTX-M, blaTEM, blaSHV genes were not detected in 1.4% (n=7) of extended spectrum beta lactamase producing Enterobacterales isolates. The blaCTX-M genes were found in 84.9% K. pneumoniae, 89.8% E. coli and 42.6% Enterobacter cloacae. The blaCTX-M belonged to clusters blaCTX-M-1 (88.8%), blaCTX-M-9 (14.8%) and blaCTX-M-2 (0.2%). The percentage of isolates with the genes of two various blaCTX-M clusters was 4.1%.

Considering the results of molecular and genetic studies with blood/bone marrow samples, a group of patients with Ph-negative myeloproliferative diseases and Jak2 V617F mutation was isolated. The quality of collected DNA/RNA samples was confirmed during molecular studies of Jak2V617F, MPLW515L/K driver mutations and mutations in the CALR exon 9 mutations. The collected samples were kept in freezers at -60 °C as per the protocol of the studies.

Disorders in region 3q26 accompanied by changes in EVI1 expression are characteristic of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Transfer of GATA2(G2DHE)/3q21 enhancer to the EVI1 promotor region and subsequent activation of EVI1 expression are fundamental molecular events that form the basis of inv(3)/t(3;3). EVI1 abnormal expression occurs when chimeric genes EVI1/RUNX1, EVI1/ETV6 are formed as a result of t(3;21)(q26;q22) и t(3;12)(q26;p13) translocations respectively. Possibilities of a standard cytogenetic testing (SCT) are limited due to a low resolution of this method. Molecular and cytogenetic methods such as fluorescent in situ hybridization (FISH), multicolor fluorescent in situ hybridization (mFISH), and multicolor high-resolution chromosome identification (mBAND) make it possible to identify complex chromosomal disturbances, marker chromosomes, submicroscopic deletions and translocations with locus deletions of known and potential genes that participate in the pathogenesis of the disease. 3q26 defects were found in 1.8% of patients with MDS and 5.6% of patients with AML (28 patients: 14 males and 14 females, the mean age 45.6 years) during a standard cytogenetic assay of bone marrow cells of patients with MDS (n=661) and AML (n=284) (except for the cases of acute promyelocyte leukemia) from January 2014 to October 2018. Defects in the EVI1 gene locus were found in 1% of patients with MDS and 3.8% of patients with AML using the standard cytogenetic assay and FISH assay. It constitutes 76% of all 3q defects (n=28) in patients with MDS/ALL within the mentioned period. inv(3)(q21q26) and t(3;3)(q21;q26) were confirmed in all cases detected during a standard cytogenetic assay with the FISH method. T(3;3)(q21;q26) with 5`EVI1 deletion and inv(3)(q21;q26) with 3q21/RPN1 deletion were detected, each on one occasion. Excluding these two cases, the rupture points at the 3q26/EVI1 region were typical. In one patient with AML, an extremely rare t(3;3)(p24;q26) translocation with rearrangement within the 5′ EVI1 was detected. True monosomy 7 was detected in six patients with inv(3)/t(3;3) In five patients, t(3;21)(q26;q22) was detected, in one of them without involvement in the translocation of the EVI1 and RUNX1 gene loci. In the standard cytogenetic assay of peripheral blood lymphocytes in remission, it was found out that the patient had a constitutional pattern of chromosomal abnormality. Four patients with AML had t(3;5)(q?25-26;q34), no EVI1 rearrangement was detected in three cases, whereas in one case, there was no sufficient specimen to perform the FISH assay. t(2;3)(p21;q26) translocation with the EVI1 gene involvement was detected in one case. One patient had

t(2;3)(q24;?q25-q26) without the EVI1 rearrangement. No similar cases have been described until now. It was impossible to perform mBAND to ensure more exact identification of translocation involved regions due to insufficient examined specimen. We suggest that all patients with 3q defects should have the FISH assay to determine whether there are rearrangements in the EVI1 gene locus. When non-typical rupture points are found, mFISH and mBand methods should be used to provide a complete characterization of the aberration structure and conduct a further study of these patients. If it was found during the standard cytogenetic assay that all BM cells have balanced translocations with 3q26 involvement and without EVI1 rearrangements during the FISH assay, it is necessary to conduct a standard cytogenetic assay of peripheral lymphocytes in remission to exclude hereditary abnormality.

The rate of oncohematological diseases in patients with constitutional rearrangements of autosomes and sex chromosomes are scarce in literature and contradictory. Hereditary disorders occupy an important place in human pathology. The majority of these disturbances are associated with numerical or structural aberrations of the whole genome or separate chromosomes. Genetically balanced chromosomal aberrations (translocations, insertions, inversions) do not commonly influence the carrier's phenotype. Chromosomal mosaicism seen in chromosomal disorders is described for sex chromosomes and multiple autosomes. High rate of leukemia in some hereditary disorders is proved during numerous studies. Clinical symptoms of Fanconi's anemia are directly associated with chromosomal instability, as there is a high risk that malignant tumors can be developed in these patients. It can be suggested that constitutional rearrangements result in the chromosomal instability of the region; genes localized within the chromosomal breakpoints are deprived of a regular orientation and can probably change their functional activity. Constitutional structural aberrations with end-points, which are similar to leukemia-associated end-points, were recorded in patients with onco-hematological diseases. This report includes data on the absolute number of constitutional rearrangements in patients of the NMRC for Hematology based on the results of a standard cytogenetic study for 2018. A total of 1,085 patients were included in the study. Cytogenetic findings were detected in 26 patients (14 females and 12 males) aged 20 to 78 years with different hematological diseases. They had a SCT of the bone marrow (BM) and the constitutional nature of rearrangements confirmed during the cytogenetic analysis of FHA-stimulated lymphocytes of peripheral blood. Six (t) translocations were detected: 3 reciprocal ones (2 in females and 1 in a male) and 3 Robertsonian translocations (1 in a female and 2 in males). Normal variants of chromosomal polymorphism were analyzed. The known rate of the found structural abnormalities with a known rate within a population was much lower than the population value. DEB test was performed in 32 patients with myelodysplastic syndrome and acute leukemia to find chromosomal instability. In 2018, no patients with chromosomal instability were

found during the DEB test.

Effectiveness of using DSP30 combined with IL2 during culturing of tumor cells in patients with CLL was assessed to detect the aberrant karyotype. Two series of cultures were tested with addition of DSP30 oligonucleotide division stimulator and interleukin 2 (DSP30+IL2) and using a standard combination of LPS and TPA (LPS+TPA). 32 patients with CLL were analyzed. They had a SCA and FISH performed at the Laboratory of Karyology of the NMRC for Hematology of the Ministry of Health of Russia from January 2018 to December 2018. Of them, 21 males and 11 females were 35 to 81 years of age (median of age was 61 years). 3 patients had t(11;14)(q13;q32) translocation. One patient was diagnosed with diffuse large-cell lymphoma. Another patient had splenic marginal zone lymphoma. The patients were excluded from a subsequent analysis. A total of 27 patients with CLL were included into the trial. 19 patients were examined prior to therapy, whereas 8 patients had a resistant and recurrent course. In the result of cultivation with DSP30+IL2, the SCA was successfully performed in 21 (78%) patients. The aberrant karyotype was found in 11 (41%) patients: one of them had one aberration, 3 (11%) had two aberrations, and 7 (26%) had three or more aberrations (complex karyotype disturbances). A normal karyotype was detected in 10 (37%) patients. 6 of them had 13q14deletion found during the FISH assay. In two cases with lacking mitoses in the DSP30+IL2 culture, chromosomal disturbances were detected in the LPS+TPA culture. In the result of cultivation with LPS+TPA, the SCA was successfully performed in 23 (85%) patients. The aberrant karyotype within the LPS+TPA culture was detected in 9 (33%) of cases: two in 4 (15%) cases and complex karyotype disturbances in 6 (22%) cases. 14 (27%) patients had a normal karyotype. 2 (7%) patients with normal karyotype when LPS+TPA were cultivated with DSP30+IL2 had clonal chromosomal aberrations. When the results of karyotyping were compared, it was found out that complex karyotyping was the most common in cultivating with DSP30+IL2 in 7 (26%) patients. 13q14 and 17p13 deletions were found in 3 cases with the help of FISH assay. Based on the SCA results, they were accompanied with balanced or imbalanced translocations within the loci. Thus, SCA is an important method used to specify the structure of chromosomal disturbances, detect additional aberrations, and, most importantly, identifying the group of patients with the highest risk of CLL, i.e. complex karyotype disturbances that help to determine the treatment strategy.

Topic 4. Study of the relationship between the structural state and the mechanism of action of new hemostatic and anticoagulant agents

New chitosan-based composition solutions were developed and prepared during the reporting period. Solution of fibrin monomer, sodium alginate, and carageenan with various solvents (AD, 0.5% AA, 0.5% UA) were introduced into its structure. They were used to make 20

various samples of application sponges. 1,284 experiments were conducted: 64 in vivo experiments, 900 in vitro experiments and assessment of physical and chemical properties of 320 measurements. In general, the results revealed a wide range of changes in hemostatic reactions both in vitro and in vivo. All the studied samples of sponges based on natural polymers in various solvents, with fairly short-term contact with blood (within 1 minute), accelerated the formation of a primary blood clot on the contact surface, but the formed clot had an insufficiently dense structure and therefore, when applying sponge-shaped samples to the wound surface of the animal's liver, it was quickly washed off by strong blood flow, except for samples in in the form of a chitosan-based sponge with the addition of a fibrin monomer to its structure. It was shown that sponges that use distilled water (pH 7.57±0,30) as solvent produced little effect on the content of basic blood coagulation substrates such as thrombocytes and fibrinogen after they contacted blood. The situation was different when sponges were made using acidic environment (pH 2.90±0.02). The effect on the hemostatic activity of the polymer mass fraction, density and total porosity of the studied sponges was proven. To further clarify the relationship between the structural state and the mechanism of action of new hemostatic agents, it is necessary to expand research with an increase in the mass fraction of the studied polymers and the introduction of another polymer based on bacterial cellulose. Cellulose sulphate with 1.3 degree of sulphation and antocoagulant activity (AC) and hydrochloride cellulose aminodesoxybutyl with molecular mass of 15-25 kDa (0.9-1.0 degree of substitution), AC and antiplatelet activity can be used to obtain thromboresistant surfaces for biomaterials. Prospects for the use of cellulose conjugate with dibornol and deoxycellulose with MM 33 kDa (degree of substitution 1.0 and 1.5) in designs for drug delivery are associated with the continued evaluation of their effect on human erythrocytes.

Topic 5. Study of immunomodulating properties of multipotent stromal cells to increase effectiveness of their use in the clinical setting to treat and prevent acute graft-versus-host disease

As a result of a prospective randomized clinical trial, it was shown for the first time that the administration of MSCs obtained from a hematopoietic cell donor at the time of restoration of the total number of leukocytes to 1*10 9 / l significantly increases the overall survival of patients after transplantation of allogeneic hematopoietic stem cells. The trial included 147 randomized patients. 69 patients were administered MSCs for the prevention of GVHD, and 78 were included in the control group. Overall survival was significantly ($p< 0.05$) higher in patients who received, in addition to standard prophylaxis, MSCs. Relapse-free survival was also higher in this group of patients. The totality of the data obtained indicates that this effect is associated with a decrease in the likelihood of relapse and the development of GVHD. Bone marrow was a graft source in 105

patients (49 patients in the group of MSC and 56 patients in the group of standard prevention). MSCs for such patients were obtained from the bone marrow of a hematopoietic cell donor. Overall and relapse-free survival in the MSCs administration group was significantly higher than in the control group. The probability of developing acute GVHD in the MSCs administration group was lower, but not significantly. The introduction of MSCs from a third-party donor did not have a similar effect.

Topic 6. Determination of clinically relevant minor histocompatibility antigens in HLA-identical hematopoietic stem cell transplantation

A cohort of healthy donors was genotyped with the developed genotyping method. The rate of occurrence of every polymorphism in the Russian population was determined. A set of peripheral blood samples taken from healthy donors who were homozygotic by the non-immunogenic variant of polymorphism was created.

Topic 7. 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

The main objective of the project is to analyze the state of stromal precursors from the bone marrow of leukemia patients at the time of diagnosis of the disease, during chemotherapy, and in the case of subsequent bone marrow transplantation.

A comparative analysis of collected samples was carried out using the level of fibroblast colony-forming units at diagnosis and at all bone marrow assay points in patients with AML, ALL, CML and DLBCL. The level of bone marrow fibroblast colony-forming units was significantly reduced at diagnosis in patients with AML and ALL, whereas the interval required to form the confluent monolayer after the primary BM transplantation was increased for any examined nosology (AML, ALL, CML). The aggregate cellular products were not significantly different from those observed in the MSC cultures of healthy donors. 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 evident that development of the examined leukemia changes the stromal precursors in the BM of patients. The level of fibroblast colony-forming units is decreased at onset and restored during therapy. However, it does not reach the values of healthy donors of the corresponding age. Patients have significantly increased developmental potential in fibroblast colony-forming units. In patients with acute leukemia, it decreases with treatment, while in patients with CML it increases even more. The total cellular production of MSCs at the onset of the disease is reduced in patients with acute leukemia and increased in patients with CML. Meanwhile, expression of proinflammatory factors in MSC is significantly increased at onset and remains high. Expression of differentiating factors

is decreased at onset and continues decreasing during therapy. It is even worse with hematopoiesis regulating factors. Their expression is not significantly changed at onset and gets decreased ten-fold when therapy is provided. It is obvious that the quantitative and qualitative characteristics of progenitor cells of the stromal microenvironment in the onset of leukemia change under the influence of tumor cells. During treatment, the quantitative characteristics of the progenitor stromal cells are restored, however, the qualitative characteristics suffer even more from the effects of therapy and are not restored in the studied follow-up periods. The function of maintaining hematopoiesis is impaired in patients with leukemia at all the studied stages of therapy.