SUMMARY OF FINAL ANALYTICAL REPORT

Topic I: study of molecular biological and biophysical mechanisms of pathological processes in diseases of the blood system

A search for pathogenic variants of von Willebrand genes, fibrinogen, blood coagulation factors V, VII, VIII, IX, X, XI and XII was performed. Novel mutations (vWF - 4, FGB -4, FGG - 5, F5 - 2, F7 - 4, F8 - 5, F9 - 1, F10 - 2, F11 - 3, F12 - 3) that were not previously described were found for all studied genes. Major disturbances in the vWF (c.2430delC), F7 (c.1391delC), F12 (IVS13 -1 G>A, -57 G>C, -62 C>T) and FGG (IVS6 +1 G>A, Arg301Cys) genes were detected. Their testing can serve as a basis for the creation of cost-effective express systems for the molecular diagnosis of Willebrand's disease, Hageman's disease, hypoproconvertinemia and fibrinogenemia.

A molecular study of ABO genotypes was conducted. RBC and sera of 6,058 patients with blood diseases and 296 healthy people were examined. Based on direct DNA sequencing of patients with polytransfusion, ABO*A101 reference allele and ABO*A102 allele were found in 9 patients and 1 patient respectively. ABO*A112 hybrid allele was seen in one more patient. People with these alleles had A1 RBC phenotype.

93 patients underwent a molecular study, including 82 patients with weak expression of antigen A. 58 of those examined had 12 variants of O-allele: 8 variants with specific c.261delG/N deletion (O101 – 60,35 %, O103 – 1,72 %, O117 – 5,17 %, O119 – 1,72 %, O201 – 8,62 %, O202 – 1,72%, O209 – 1,72 %, O210 – 10,34 %), 4 variants of hybrid alleles, three of which having the c.261delG/N deletion (O111 – 1,72 %, O205 – 1,72 %, O208 – 1,72 %) and one non-deletional O303 allele (3,45 %). 5 ABO*A allele polymorphisms (A201 – 85,88 %, A205 – 5,88 %, A206 – 1,18 %, hybrid allele A209 – 1,18 %, AW.06 - 2,35 %) were detected among 82 patients with a weak expression of antigen A. Phenotypically, A201 allele and A209 hybrid allele were manifested as antigen A2, A205 allele combined with O303 was manifested as A2 and combined with O101 as A3, A206 allele combined with O303 was manifested as A3, whereas expression of AW.06 alleles on RBC membranes was very weak. In two people with posttransfusion chimerism, having the ABO*A101B101 genotype, the A2 antigen was identified by serological methods.

A mathematical model of platelet activation under conditions of unsteady shear stresses and a mathematical model describing thrombosis of the capillary bed and vascular plexuses have been created. Numerical modeling of dynamic clustering processes of receptors associated with platelet activation was carried out. Using in vitro experiments, the relationship of platelet activation with VWF concentration and shear rate was studied.

Topic II: study of immunogenicity of variant peptides encoded by human genomic polymorphisms

Protocols of getting and differentiating activated dendrite cells loaded with synthetic peptides and derived from peripheral monocytes were mastered. The protocol obtaining antigen-specific T-cell expansion from naive T cells cultivated with autologous activated dendrite cells was mastered. The protocol assessing expansion effectiveness with the method of flow cytometry and MHC tetramer was mastered. A panel of 7 antigens coded by polymorphic human genes (minor antigens of histocompatibility). Healthy donors the cells of whom were used to deliver antigen-specific T cell expansion were genotyped and selected. The rates of naive antigen-specific precursors were subsequently evaluated. Antigen-specific cells were selected, the repertoires of T cell receptors were sequenced and analyzed.

Topic III: study of molecular, cytogenetic and immunomorphological basis of pathogenesis of clonal diseases of the blood system

The rate of rearrangement of immunoglobulin genes was determined in patients with the primary mediastinal large B-cell lymphoma (PMLBCL) and its stability at recurrence was determined. The overall detection rate of B-cell clonality in PMLBCL was 79.3%. In 2 patients who achieved complete remission, metachronous development of grey area mediastinal lymphoma was detected, whereas a shift/disappearance of initial clonal rearrangement of immunoglobulin genes was detected during a molecular and genetic study. A tumor clone identical to the one at the onset of the disease was found in 2 more patients at an early onset of the disease. Thus, molecular genetic studies have confirmed the preservation of the original clonal rearrangements of immunoglobulin genes in the development of early relapses of the disease and refute the clonal relationship of the tumor in the metachronous development of mediastinal lymphoma of the gray zone.

The properties of bone marrow stromal precursor cells such as multiponent mesenchymal stromal cells (MSC) and fibroblast colony-forming units (FCFU) were compared in patients with diffuse B-cell lymphoma (DBCL) without damage of the bone marrow and in healthy donors. The properties of MSC at onset significantly differ from those of healthy donors. For example, total cellular production is significantly higher in primary patients as compared to donors. Cellular parameters of MSC were changed, the average fluorescence level (AFL) of ICAM1 adhesion molecule was increased on the cellular surface. The AFL of MSC- HLA-ABC, CD73 and CD90 markers were significantly increased. The relative expression level (REL) of the MP4, MMP2, FGFR1, ICAM1 genes is decreased at MSC, and increased at FGFR2. Thus, in spite of the lack of proved bone marrow lesion, patients with DBCL have changed components of the stromal

microenvironment regulating hematogenesis.

The samples of lymph nodes, blood and bone marrow were studied to detect expression of the cancer-testicular PRAME gene. PRAME gene activity was observed in 21 (60%) patients. It turned out that PRAME hyperexpression was associated with common diseases, bone marrow lesion, bone marrow involvement, tumor leukocytosis, worse values of total and event-free survival including following transplantation of hematopoietic stem cells.

A method of allele-specific PCR in real time was developed to detect point mutations in the STAT3 p.Y640F; p.N647I; p.D661V; p.D661Y; p.D661H; p.D661N gene. High specificity of the method was shown during differential diagnostics in patients with T-cell large granular lymphocyte (LGL) leukemia. The method was superior to lymphocyte immunophenotyping and determination of T cell clonality by rearrangement of the TCRG and TCRB genes. It was necessary to implement the method of allele-specific real-time PCR to detect point mutations in the STAT3 gene to the protocol of primary examination of patients with suspected T-cell LGL leukemia.

2 predictive HLA-alleles were detected in CLL with non-mutated IGHV genes with 2 more of them being found for the most aggressive variants of this disease. Moreover, some differences in HLA alleles repertoire were observed among patients with expression of IGHV genes belonging to various families.

A method for determining mutations in the BTK gene based on AS-PCR has been developed, which is significantly simpler and cheaper than ddPCR, NGS and others, but no less sensitive and reliable. The proposed test system should be used for early detection of resistance in patients with CLL who are being treated with ibrutinib, which will allow timely correction of therapy tactics.

Molecular and genetic studies of mutations in the TET2, ASXL1, Dnmt3a, SF3B1 and SRSF2 genes were developed and implemented into the laboratory practice. Over 60 patients were examined to detect mutations in the ASXL1, SF3B1 and SRSF2 genes. Statistical treatment of the obtained data related to the study of mutations in the ASXL1 and SF3B1 genes was initiated. As soon as it is over, the results will be published in a magazine. The detected variety of combined mutations in the ASXL1 and SF3B1 genes, and their association with clinical variants of MDS makes it possible to predict the molecular response to specific therapy.

A molecular and cytogenetic study (FISH) of patients with multiple myeloma (MM) at the disease onset was carried out. Trisomy 5, 9 and 15 was detected. The MM recurrence was accompanied with amp1q21 when hyperdiploidy is preserved. Prior to therapy and at the onset, c.182A>C (p.Q61P) heterozygous clonal mutation was detected in the N-RAS gene. The mutation disturbed the regulation of MAPK signaling path. RNA-seq transcriptomic analysis displayed an increased expression of the IL6 gene in case of recurrence (by 30 times). It could serve as a releaser of MM progression as the cytokine

can promote cellular proliferation by activating various signaling paths (MAPK, JAK-STAT, PI3K). Disease progression was also accompanied by more severe expression of key regulatory genes (c- MYC, Notch2, MDM, RAF1, STAT4, mTOR) with a steep fall of expression of immunoglobulin genes resulting in deep immunodeficiency. Summarizing the data, we can conclude that complex molecular diagnostics should be used in MM. It includes full-scale search, expression analysis and mutational analysis of a wide specter of regulatory protein genes. It is found out that 30 patients with MM and cast-nephropathy who required dialysis had renal response only when the hematological response to the first-line chemotherapy was achieved. Grade 2 interstitial fibrosis was detected in the majority of patients by the beginning of the therapy. It was determined that the rate of interstitial fibrosis in the renal specimen was 40%. By the beginning of the therapy, it is an unfavorable prognostic factor for reversibility of an acute renal failure.

The study of minimal residual disease (MRD) within the ALL-2016 therapy protocol included 3 control end-points such as Day +70 (induction 2), Day +133 (consolidation 2), and Day +190 (consolidation 4). At day +70, +133 and +190, the study was performed in 57, 45 and 34 patients. At day 70, 4 patients had no clinical and histological remission (CHR), whereas a number of tumor cells amounted to 21.5%, 8.24%, 7.76% and 0.138%. Out of 53 patients with CHR, 32 patients (60.4%) were MRD negative, whereas 21 patients (39.6%) were MRD positive. In the group of MRD positive patients, the MRD median was 0.07% (0.018%-0.661%). At day +133, 34 (75.6%) patients of 45 had a MRD negative status (25 had a MRD negative status at day 70, 7 had a MRD positive status at day 70, and two patients had no study of MRD done at day 70). At day 133, 11 (24.4%) patients had residual tumor cells. Their median was 0.007% (the interquartile range is 0.002%-0.071%). At day 70, MRD was detected in 10 patients. Within the group of patients, the percentage of tumor cells decreased by day 133 ($p = 0.0004$). At day 190, 31 (91.2%) of 34 patients had MRD negative status (4 of them were MRD negative, i.e. MRD was determined at day 133). 3 patients had MRD at day 190 (just like at day 133). In 2 of these patients, MRD was examined at month 9 (maintenance therapy stage). No tumor cells were detected by that time. No statistically significant changes were detected in the percentage of tumor cells during days 133-190 ($p = 0.109$).

Among A. baumannii (n=74) isolated from the hemoculture of patients with tumors of the blood system, carbapenem-insensitive isolates prevailed (n=54; 74.3%), of which 70.9% (n=39) contained genes of acquired OXA-carbapenemases in their genome. The most common genes of acquired OXA-carbapenemases included blaOXA-24/40-like (51.3%).They were followed by the genes belonging to groups blaOXA-23 (38.5%) and blaOXA-58 (10.3%). OXA-24/40 carbapenemase-producing A. baumannii isolates had higher MIC50/90 values of carbapenems as compared to blaOXA-23-like gene isolates. The molecular and genetic study of E. coli isolates producing extended spectrum beta lactamases isolated from the intestinal mucosa demonstrated the lack of genetic affinity between

isolates isolated at first admission to the hospital and the presence of genetic similarity in 59% of isolates isolated during cytostatic therapy and hospital stay. The study proved the possibility of exogenous transfer of E. coli isolates from one patient to another during their stay in the hospital.

Topic IV: study of interaction between the structural state and mechanism of action of new haemostatic and anticoagulant agents

At the stage of the state program implementation, the outcomes include development of new coatings with 1.5 and 2.0% solutions of kappa-carageenan, 2.0 and 3.0% solutions of sodium alginate, 1.5 and 2.0% of chitosan and bacterial cellulose solutions, and a complex analysis of hemostatic, physical and chemical properties of local wound sponge-shaped coverings in vivo and in vitro. The in vitro study was conducted using the suggested test system (patents of the Russian Federation No.2701195 and No.26950756). 43 various samples of application sponges were made. 2,187 experiments were conducted: 68 in vivo experiments, 1,296 in vitro experiments and assessment of physical and chemical properties of 823 measurements. During in vivo and in vitro experiments, it has been found out that kappa-carageenan-based coverings have a better haemostatic activity. In particular, it was stated that the content of kappa-carageenan within a sponge at neutral pH

- produces no effect on the PLT count at a concentration of 0.5-1.5%, but results in their decrease by 64.8% (from 179.0 to 63.0 ×109/L according to the median);
- produced no effect on the level of fibrinogen at the studied concentrations (0.5-2.0%);
- as per thromboelastometry, it results in higher (after the sponge is contacted) coagulation, which is the greatest when the concentration of carageenan reaches 2.0%;
- increases the rate of thrombin creation and generation intensity irrespective of carageenan concentration.

Despite the ligand, a satisfactory strength of direct-acting anticoagulant application to the surface of polymer for medical purposes made it possible to achieve thromboresistance. An optimal dose of cellulose sulphate (degree of polymerization - 120, degree of sulfatation - 1.3-1.8) was determined to achieve the maximum antithrombotic effect during intravenous administration to guinea pigs. During in vitro experiments, some perspective compounds, which can be used as components of developed constructions to deliver medicinal products were selected based on the structure-hemocompatibility association. They included lignin sulphates with low antithrombin activity (decreased ADP-induced aggregation of PLT), sulfoethyl derivatives of chitosan and sodium taurinate (< 0.005 mg/ml; no destruction of red cell membrane), hydroxyethyl starch with molecular weight of 200,000 (5·10-6 mg/ml; produced no effect on plasma coagulation and human PLT aggregation).

Topic V: optimization of programmatic hemoblastosis chemotherapy based on patient-specific biological markers of the disease

Timely intensification of CML therapy in case of its failure, according to the criteria of modern recommendations, makes it possible to obtain results comparable to those when using TKI2 in the first line. This is how the maximum effect is achieved through use of every TKI therapy line accompanied with low treatment costs and less risks of CML progression and development of TKI2 adverse effects. The high frequency of achieving deep MR in the future will increase the proportion of patients in remission without treatment, which is of no small economic importance in the conditions of a gradually increasing population of CML patients.

The extent of a tumor (especially the presence of bulky), FLIPI index and FL morphology are the key criteria of first-line therapy selection. As a half of patients with resistant FL have 3A and 3A+3B (55%) cytological type and diffuse growth pattern (55%), R-CHOP is basically selected. The R-B scheme is more effective in FL of the 1^{st} and 2^{nd} cytological type.

Targeted drugs are widely used today to treat oncohematological patients. For the treatment of T-ALL/T-cell lymphoblastic lymphoma patients it is promising to study the use of nelarabine in the first line of therapy, which may improve the results of treatment after the first line and reduce the number of patients who need anti-relapse therapy, which is still ineffective. Use of nelarabine in the clinical setting improved the survival indicators of patients with resistant/recurrent T-ALL/T-cell lymphoblastic lymphoma. However, a few available long-term observation outcomes obtained following all-HSCT are not very optimistic. Currently, an urgent problem is the development of not only highly effective anti-relapse therapy regimens in patients with a resistant course/relapse of T-ALL/T cell lymphoblastic lymphoma but also improving the results of therapy in the first line of therapy and, possibly, the use of new approaches to the treatment of patients after allo-HSCT.

In the five years that have passed since the first publication of the results of AML treatment at the coordination center, despite a significantly larger number of patients (n=173), conclusions about the main factors affecting the outcomes of therapy in AML patients aged 18 to 60 years have not changed. The initial group of risk assessed based on the outcomes of a cytogenetic study, time to complete remission (achievement of CR following the second course of therapy is an extremely unfavourable factor) and all-HSCT during the first CR are essential. All patients with AML will go through transplantation of allogenic bone marrow if HLA-compatible donor is available (no later than 6.5 months from CR achievement). A lack of MRD following the first course of induction therapy is probably the only exception from all-HSCT indications during the first CR.

Topic VI: optimization of the program of diagnosis, treatment and monitoring of non-tumor orphan diseases of the blood system in adults based on molecular-genetic, biochemical, immunophenotypic parameters

Radiation criteria for assessing the damage of the osteoarticular system in adult patients with Gaucher type I disease have been developed.

A standard image of radiation injury of bones is described. A modified scale assessing the severity of bone and joint lesions in adult patients with type I Gaucher's disease is suggested. X-ray semiotics and MR-semiotics of typical changes in bones and joints in patients with Gaucher's disease is described. A specter of detected radiation changes is subdivided into 2 principal groups such as reversible and irreversible changes.

Radiation criteria of bone lesions were developed and implemented with the use of special high-tech MRI methods, which allow to optimize the control of pathogenetic therapy effectiveness in Gaucher's disease.

The parameters of controlling the effectiveness of Gaucher disease treatment have been determined: pathogenetic therapy leads to regression of reversible bone changes, can prevent the development of irreversible damage to the bone and joint system, but is not able to eliminate existing orthopedic defects.

An algorithm of complex radiation examination of adult patients with type I Gaucher's disease was created to detect bone and joint lesions and assess the rate of their severity.

A unique method of detecting deficiencies of blood clotting factors by thromboelastometry has been developed and tested. A patent for the invention has been obtained. The genetic base of patients with rare hereditary coagulopathies continues to be replenished. Data analysis makes it possible to characterize the spectrum of genetic disorders and identify the most common or new mutations. From a clinical point of view, genetic studies make it possible to verify the diagnosis and prescribe appropriate therapy.

Topic VII: improvement of various stages of allogeneic and autologous hematopoietic stem cell transplantation and development of new approaches to prevention and therapy of post-transplantation complications

The interaction between MSC and peripheral blood lymphocytes in healthy donors was analyzed. Lymphocytes and MSC were cultivated at 37°C and 5% CO2 for 4 days. Lymphocytes cultivated without MSC were used as control samples. It was shown that donor MSC are divided into 2 groups. In one group, MSC have no significantly increased HLA-DR expression in co-culturing with lymphocytes, whereas expression of the MHC antigen has risen significantly. A detailed analysis will be the subject of further

investigation, as division into 2 groups can matter in assessment of MSC clinical effectiveness for prevention and therapy of acute graft-versus-host disease and will allow the use of the most effective samples in clinical practice. The outcomes of repeated all-HSCT performed at the National Medical Research Center for Hematology of the Ministry of Health of the Russian Federation were analyzed. Following recurrent all-HSCT, OS and event-free survival were 38.5% and 27.6% respectively. Based on the data obtained in this work, the change of the donor has little effect on the results of repeated allo-HSCT. Thus, a prolonged search and activation of another unrelated donor seems unjustified, especially if the reason for repeated allo-HSCT is primary non-graft implantation, accompanied by pancytopenia, agranulocytosis and a high risk of infectious complications. In this case, it may be justifiable to have allo-HSCT with another - haploidentical - donor. Hematopoietic stem cell should be used as a graft source. It gives the possibility to prepare many precursor cells and restore hematopoiesis rapidly. It is noted that the second allo-HSCT is associated with a high risk of life-threatening complications. They are mainly due to the need of pre-transplantation conditioning, immune suppression against the background of severe somatic status and infectious complications. This is the reason for high mortality (61.5%), especially in the early period (the first 100 days) after transplantation, including the cases with restored hematopoiesis.

A method for determining minimal residual disease in patients with multiple myeloma after autologous HSCT by PET-CT using two radiopharmaceuticals has been studied, since this method has a high sensitivity (90%) and specificity (75%) for determining plasma cell damage. Moreover, whole-body PET/CT allows to detect bone and extramedullary plasmocytes in patients with MM. It is found out that 11C-methionine absorption activity with myeloma cells exceeded a similar value by about 5 times when 18F-FDG was used. The use of 11C-methionine to detect minimal tumor lesion in MM patients provides additional opportunities to assess the antitumor response compared to 18F-FDG.

Effectiveness of autologous transplantation was studied in patients with multiple myeloma and dialysis-dependent renal failure. The study allowed to improve the antitumor response (71% PR, 29% VGPR) and achieve the minimum renal response in 14% of cases. It is possible to stop hemodialysis in patients with minimal renal response. Patient follow-up is should be continued. There is no need for renal replacement therapy for 24-100 months. High values of progression-free survival and total survival based on the present study results were obtained and amounted to 59% and 93% respectively with the observation median of 53 months. In accordance with the present results, low transplantation-associated mortality values can be explained by improved diagnostic methods and accompanying therapy.

The negative effect of mutations in the TP53 gene on cell lymphoma therapy electiveness was studied in accordance with the MCL-2016 protocol results because there

are a limited number of studies about the effect produced by mantle cell lymphoma therapy effectiveness on the TP53 gene mutations and about effective therapy for this group of patients. It is shown that the high-risk group with blastoid MCL morphology, complex karyotype, hyperleukocytosis and del 17p requires a molecular and genetic study to detect mutations in the TP53 gene; it is also necessary to develop methods of high resolution molecular diagnostics with a free tumor DNA plasma to make detection in the TP53 gene in MCL more exact. If a mutation in the TR53 gene is detected, it is necessary to search for a compatible bone marrow donor/hematopoietic hematopoietic stem cells. In the presence of a compatible donor, the absence of clinical remission and the positivity of the MRD after CT, allo-HSCT is indicated. In patients without a mutation in the TR53 gene, the use of the MCL-2016 protocol showed high efficiency in achieving remission, MRD-negativity, with acceptable toxicity and the possibility of harvesting auto-HSCT, which dictates the need to increase the number of patients included in the study and the follow-up period for the formation of final conclusions. It is necessary to develop alternative therapy options for patients with MCL with mutations in the TR53 gene, using combined targeted therapy and CT.

A study of effectiveness of the new strategy of induction and high-dose chemotherapy of primary diffuse large B-cell lymphoma of the central nervous system was conducted. Intermediate results of the CNS-2015 protocol. The goal of induction therapy was to achieve not long-term remission due to intense chemotherapeutic effect, but the most rapid kinetic tumor reduction, restored neurological status and possibility to prepare sufficient volume of haemoblasts. Better values of disease-free survival (DFS) and total survival (TS) were found in patients with CNS PDBCL after introduction of myeloablative conditioning regimens with high doses of thiophosphamide, busulfan and cyclophosphamide (TBC mode) into protocols with subsequent transplantation of autologous hematopoietic stem cells. Use of the approach makes it possible to achieve a long-term event-free survival in 90% of patients with an extremely unfavorable variant of the disease.