# SUMMARY OF FINAL ANALYTICAL REPORT

## Topic I: study of molecular, biological and biophysical mechanisms of implementation of pathological processes in blood system disorders

During implementation of the project, the purpose of which was to conduct molecular and genetic research of common and rare coagulopathies in Russia, mutation analysis systems were developed and a search for pathological variants for the VWF, FGA, FGB, FGG, F5, F7, F10, F11, F12 and F13 genes was carried out. The mutation spectra were determined, which were highly diverse for all the studied genes, with the exception of the F12 gene. The largest number of different mutations (33 and among them 11 new ones) was found for the VWF gene. The major microdeletion was c.2430delC, which is also common in a number of European countries and is most often found in patients with type 3 Willebrand's disease. This microdeletion occurred in 32% of patients. Major abnormalities were also found in the *FGG* (IVS6 +1 G>A и Arg301Cys) and *F7* (c.1391delC) genes. In patients with Hageman's disease, only 6 different mutations were found in the F12 gene with a clear dominance of three of them, IVS13 -1 G>A splicing mutations and two nucleotide substitutions in the promoter region at positions -57 and -62. Three other mutations (c.614delC, IVS4 -1 G/A and Tyr218His) were detected once that had never previously been encountered in the global population. New mutations (44) were also identified in the *FGB*, *FGG*, *F5*, *F8*, *F10*, *F11* and *F13* genes. Apart from traditional mutations, a new complex genetic abnormality was detected in the *vWF* gene. It was due to the interaction between a gene and a pseudogene found on different chromosomes and a very rare nucleotide substitution in the *F5* gene intron responsible for a highly effective abnormal splicing site. Detection of dominant disturbances in the *vWF*, *FGG*, *F7* and *F12* genes made it possible to offer cost-effective express systems for DNA diagnostics of von Willibrand disease, Hageman factor, hypoproconvertinemia and fibrinogenemia. The study of a genetic predisposition to inhibitory hemophilia A has shown that poor prognostic factors can include both severe mutations in the *F8* gene and pathogenic variants in many genes of regulatory proteins. A study was also conducted that showed an association of intense pain in patients with hemophilia A and B with the G allele of polymorphism (rs1799971) of the OPRM1 gene.

A rare allelic variant of RhD weak type 122 was detected. Its serological characteristics were examined. The immunogenetic features of *ABO*O* alleles were studied in individuals of Russian origin. The rate of alleles and genotypes of *ABO*A2* were studied. A rare non-deletion *ABO*O.01.26* allele with residual A-glycosyltransferase activity was described in a patient with acute hemolytic complication after transfusion of one-group plasma. Original PCR systems, which made it possible to carry out a discriminant analysis of rare and search for new allele variants of highly homologous *RHD* and *RHCE* genes associated with Rh antigen abnormalities were developed and successfully approved. The introduction of molecular methods of studying the genes of erythrocyte systems into the laboratory of transfusiological immunohematology made it possible to resolve ambiguous results of serological research methods in potential recipients with difficult to determine blood groups, to study the mechanisms of the appearance of allelic variants of different genes, to study the serological properties of the antigens or enzymes encoded by them (glycosyltransferases), and to understand the pathogenesis of posttransfusion hemolytic complications. Development of original PCR systems by the Laboratory of Genetic Engineering made it possible for a discriminant analysis of rare and search for new allele variants of highly homologous *RHD* and *RHCE* genes.

Numerical simulation of activated thrombogenesis was performed with artificial circulatory support devices developed by Food and Drug Administration, FDA: 1) FDA Nozzle and 2) FDA Blood

Pump. Basic structural features of these devices were detected. They determined the level of trombogenicity. A rapid algorithm was developed, and a program was implemented in the Python programming language. These were used to reconstruct the geometry of the studied vessels with the help of Doppler ultrasound (one-dimensional D mode). The manufacturing technology of silicone castings of personalized areas of the patient's vascular tree was established based on MRI data. A series of validation in vitro experiments were performed to control activation of thrombocytes within complex three-dimensional objects such as circulatory assist devices, arteriovenous fistulas for hemodialysis, and atherosclerosis affected vessels. The manufacturing technology of specialized microfluidity biochips modelling vascular networks was developed and adjusted. Tests for thrombogenicity were carried out. A phenomenological model of signal transduction activation in the PI3K-Akt-mTOR cascade was constructed. The parameters of the system under consideration have been found, changes in which can critically affect the lowering of the platelet activation threshold.

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

Detection of peptides within MHC molecules is essential for the formation of alloreactive T-cell immune response. Following thymus selection, cells can recognize allogenic but non-autologous antigens. However, it has been shown that not every antigen is immunogenic, i.e. capable of producing an immune response. According to our hypothesis, differences in the rate of antigen-specific T cells can be the reason for different immunogenicity.

Protocols of getting and differentiating activated dendrite cells loaded with synthetic peptides and derived from peripheral monocytes were mastered within the research project. Protocols obtaining and assessing the effectiveness of antigen-specific T-cell expansions from naive T-cells cultivated with autologous activated dendrite cells of human donors were developed on a panel with 7 antigens coded by human genes and presented within the HLA-A*02 molecule. The rates of naive antigen-specific precursors were subsequently evaluated. Repertoire sequencing of T-cell cultures was followed by the detection of 258 unique sequences of alpha- and beta-chains of receptors.

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

During the project, it was possible to obtain the data about the effect of tumor on the bone marrow microenvironment in patients with diffuse large B-cell lymphoma regardless of the intercellular contact between mesenchymal stromal cells and tumor cells. Due to the presence of changes at the molecular and cellular level, bone tissue was examined in patients with diffuse large B-cell lymphoma. At disease onset, patients with DLBCL had significantly increased levels of urinary deoxypyridinoline/creatinine and blood b-cross-laps, which indicated the activation of osteodestruction processes. Blood levels of vitamin D3 were low as compared to the control group. When patients were examined many years after treatment, all biochemical values were normalized in the presence of long-term remission of the underlying disease. There was no significant difference between the groups during densitometry in the spine and femoral neck. The possibility of whole-body diffusion-weighted MRI (WB-DW-MRI) was studied as compared to PET/CT while assessing the tumor size and prevalence and measuring bone marrow lesions (in different cytological types) in diagnostics and staging of the disease in patients with follicular lymphoma (FL). WB-DW-MRI and PET/CT showed similar outcomes while assessing the extent of tumor lesion. However, WB-DW-MRI method made it possible to obtain detailed visualization of the bone marrow of

lesions and surrounding soft tissues both in the onset of FL and in the process of monitoring the effectiveness of polychemotherapy.

Highly sensitive qualitative methods for determining point somatic mutations in the following genes were developed: RHOA c.50G>T (p.G17V); STAT3: c.1919A>T (p.Y640F); c.1940A>T (p.N647I); c.1982A>T (p.D661V); c.1981G>T (p.D661Y); c.1981G>C (p.D661H); c.1981G>A (p.D661N); STAT5B c.1924A>C (p.N642H), and gene mutations IDH1 (p.R132H, p.R132C/G/S) and IDH2 (p.R140Q; p.R172K). Specificity and sensitivity of the research of these mutations were determined in angioimmunoblastic T-cell lymphoma (AITL), T-cell large granular lymphocyte leukemia (TCLGLL), and acute myeloid leukemia (AML). A large control group of patients with various hematological disorders was examined to test specificity of the methods. The role of these studies was determined as related to the routine molecular and genetic PCR method of determining T-cell clonality by T-cell receptor gene rearrangement used to diagnose T-cell lymphomas. It was proven that it is necessary to include PCR testing of these mutations into standard protocols of management of patients with AITL and TCLGLL to diagnose, stage and assess effectiveness of anti-tumor therapy.

Significant differences in the *IGHV* gene repertoire were reported for CLL, MCL, HCL, and splenic marginal zone lymphoma (SMZL). There was a trend to stereotypy of antigenic B-cell receptors in SMZL and MCL. Though the sample was small, 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. The study of non-selective larger samples in patients with CLL might make it possible to find new associations of HLA alleles with the course of the disease. In patients with the most common stereotyped antigen receptors (SAR) in Russia, genetic disturbances were shown to occur more frequently than in patients with CLL. Meanwhile, mutations in the*TP53* and del17p13 are more common in groups CLL#1 and CLL#6 as compared to literature data. This could be due to the more sensitive methods used. Moreover, some patients within our cohort relapsed following FCR therapy. Population differences in disease progression patterns cannot be excluded as well. Further study of genetic disturbances in this and other groups of SAR will improve therapy and understanding of pathogenesis of the disease in the future. A method detecting BTK mutation was developed, which is significantly simpler and less expensive as compared to previously used methods such as ddPCR, NGS and etc, but no less sensitive and reliable.

Molecular and genetic research with the 'next generation sequencing' (NGS) method of bone marrow and peripheral blood samples in 48 patients diagnosed with 'myelodisplastic syndrome' was carried out. The most common genetic mutations were as follows: ASXL1 (ASXL1 c.1934_1943dupGTGGCCCGGG, ASXL1 c.1944T>G (?Syn), ASXL1 c.4112_4114delAGA, ASXL1 c.2644C>T, ASXL1 c.1934dupG, ASXL1 c.3306G>T, ASXL1 c.3935C>T, ASXL1 c.3593G>A, ASXL1 c.4382C>T, ASXL1 c.1934dupG and ASXL1 c.2893C>T), DNMT3A (DNMT3A c.2644C>T , DNMT3A c.2196dupT, DNMT3A c.1904G>A , DNMT3A c.917G>A, DNMT3A c.2644C>T, DNMT3A c.1082_1087dupAGCAGC) and TET2 (TET2. c.3881G>C, TET2 c.1586delT, TET2 c.3571C>T, TET2 c.2662T>C, TET2 c.2238dupA, TET2 c.703dupT). Mutations in the genes of RNA splicing coding proteins ZRSR2 (ZRSR2 c.1093G>T, ZRSR2 c.700C>T and ZRSR2 c.195_198delAAGA), U2AF1 (U2AF1 c.101C>A, U2AF1 c.101C>T, U2AF1 c.101C>T, U2AF1 c.470A>C), SRSF2 (c.284_307delCCCCGGACTCACACCACAGCCGCC, SRSF2 c.284C>A, SRSF2) and SF3B1 (SF3B1 c.2098A>G, SF3B1 c.2347G>A and SF3B1 c.1998G>C). Nevertheless, the pathogenic value of the discovered mutations of the genes of epigenetic regulation and RNA splicing requires further research.

During the research, significant differences in the studied cytometric values were discovered when the groups of donors and patients were compared; tumor cells of CLL, MCL and MZL are characterized by different expression of FAS, PD-L1, CD80 antigens, and different relative number of T-cells expressing PD-1 and PD-L1 as compared to one another and the control group. Depending on the stage of CLL, immunophenotype of tumor B-cells and T-lymphocytes in the peripheral blood have their own characteristics. In advanced stages of CLL, tumor cells express less co-stimulating antigens (CD80, CD86), and rate of T helpers exhaustion is higher in advanced stages. This is due to a greater expression of PD-1+CD4+ cells. A diversified and dynamic characterization of the functional condition of immunological synapse was provided during the conducted study in the presence of B-cell diseases with leukemization such as MCL and MZL. It is found that in case of these diseases, the immune phenotype of tumor cells and T cells expressing PD-1 and PD-L1 has certain traits and distinctive features. Unlike CLL, exhaustion and anergy of T cells is typical of MCL, whereas MZL was characterized by the exhausted function of T cells. Thus, the study demonstrates different and nosology-dependent expression of antigens of immunological synapse on tumor cells and different use by tumor cells of LPD mechanisms evading the T cellular and immune response by changing the profile of immunological synapse antigen expression.

The study included 218 patients with multiple myeloma aged 30 to 81 years old who had been observed from December 2009 to September 2020; FISH study in bone marrow was performed. The rate of primary and secondary chromosomal disturbances at the onset amounted to 87.6% and 70.7% respectively. Cytogenetic aberrations and their combination in patients with multiple myeloma should be regarded as an important prognostic factor. A statistically significant correlation between the values of OS/PFS and chromosomal disturbances was detected. 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). To predict the duration of remission, a complex molecular and genetic analysis should be performed. While examining the plasmacytoma substrate in multiple myeloma, significant differences in immunohistochemical parameters of extramedullary and bone plasmacytoma were detected. The proliferative activity of extramedullary plasmacytoma cells is significantly higher than the one of the bone plasmacytoma cells. Expression of CD166 adhesive molecule in the cells of extramedullary plasmacytoma was significantly lower than that in the cells of bone plasmacytoma. The protein most likely participated in the modelling of osteogenesis. 42 patients with newly diagnosed MM and acute renal damage due to the myelogenic cast nephropathy were included into the prospective and retrospective study. The morphological and immunohistochemical predictors of reversibility with grade 3 acute renal injury requiring dialysis in MM is a new original approach that makes it possible to predict the outcome of a renal failure. A model predicting the renal response was developed based on a set of morphometric data, intensity of epithelial and mesenchymal transformation and effectiveness of anti-tumor therapy among patients with myeloma cast nephropathy requiring dialysis.

During maintenance therapy, no MRD was found in any patient, which indicates that the ALL-2016 protocol makes it possible to achieve deep remission during induction and consolidation. The rate of MRD clearance did not depend on the immunophenotype of tumor population. Determination of MRD in patients with AML and ALL during the first complete remission prior to transplantation of allogenic bone marrow is required to stratify the risk of allo-HSCT and finding patients who will need preventive post-transplantation therapy to prevent disease relapse. The nature of distribution of Vβ families at T helpers in AA is polyclonal. Unlike T-helpers, the size of the detected TKP-Vβ clones was much bigger within the population of cytotoxic T lymphocytes (CTL). All patients with AA had an increased number

of CTL, effectory $CD4^+$ and $CD8^+$ cells, $CD4^+$ memory cells and a reduced percentage of naive $CD4^+$ and $CD8^+$cells, regulatory T-cells, and double negative T-cells ($CD3^+CD4^-CD8^-$). Different strategies of GVHD prevention did not influence the clinical outcome. The schemes of immunosupressive therapy (IST) used at our clinic were characterized by a slow restoration of T cells, especially T helpers. In case of classical IST and use of post-transplantation cyclophosphamides, T cells were restored due to CTL. While using ex vivo TCR αβ-depletion, T-cell link of the immune system was restored during the first 6 months at the expense of all studied subpopulations of memory T-cells (T naive and stem memory cells), T cells of the central memory, T cells of transitory memory, T cells of effectory memory, and T terminal effectors. While using the modes with post-transplantation cyclophosphamide and/or ATG, it occurs at the expense of effector pool. The study showed that the recovery of the studied subpopulations more than 1 month after transplantation differs significantly depending on the GVHD prevention regimen.

The study of genetic affinity of carbapenem insensitive isolates of *Acinetobacter baumannii* isolated from the blood of patients with tumors of the blood system has shown that *A. baumannii* isolated from the hemoculture of patients who have been treated at 7 Russian hospitals (2003-2017) belonged to one of the international clonal lineages (G1-G14). They were detected using two multiplex PCRs developed for selective amplification of alleles of the *ompA*, *csuE* and *bla*genes and $_{\text{OXA-51-similar genes.}}$ The genetic variety of *A. baumannii* was studied with the method of PCR of accidental polymorphous DNA fragments (RAPD-PCR) using OPA-2 primers. 96 *A. baumannii* isolates were examined. 77 (80.2%) of them were insensitive to meropenem and/or imipenem. The studied *A. baumannii* were related to 8 clonal lineages, which are common in the world. 62.3% of them belonged to the G1 international clonal lineage (international clone II, IC II). The genes of acquired carbapenemases were detected in 79.2% (61/77) of carbapenem-insensitive *A. baumannii isolates (bla*$_{\text{OXA-24/40}}$-45,9%, *bla*$_{\text{OXA-23}}$-45,9%, *bla*$_{\text{OXA-58}}$-6,6%, combination of *bla*$_{\text{OXA-24/40}}$+ *bla*$_{\text{OXA-23}}$- 1,6%). Over a half of *A. baumannii* isolates having the *bla*$_{\text{OXA-23-like}}$ genes and *A. baumannii* isolates having the *bla*$_{\text{OXA-24/40-like}}$ genes were related to G1 group (IC II) (67,9% and 64,3%, respectively). 84 RAPD-profiles were detected during RAPD-genotyping. Genetically alive *A. baumannii* (similarity coefficient (SC) ≥ 80%) were united in 20 clusters (clones) and included 85.4% of strains. A perfect match of RAPD-profiles (100% SC) was seen in 22 (23%) strains isolated from patients both within the same hospital, and at hospitals located in different cities.

Conclusion. The conducted study showed that *A. baumannii* belonged to the international clonal lineage, and the genetic variety and growth of *A. baumannii* identical clones at hematological hospitals.

The ability of *Candida* spp. to produce biofilms was determined with a spectrophotometric method and tetrazolium salt. The study included 428 isolates of *Candida* spp., with 172, 256, 361 and 67 of them being isolated from patients with blood system tumors, without blood system tumors, a hemoculture, and other sterile samples. The ability to produce biofilms was found in 41.8% (179 strains of 428) of *Candida* spp. isolated from blood and other sterile samples. A similar rate was found both in patients with (41.9%) and without (41.8%) blood system tumors. The biofilm products were more frequently detected among *C. tropicalis* (89.5 %) and *C. krusei* (75 %) as compared to *C. parapsilosis* (41.3 %), *C. albicans* (28.6 %) and *C. glabrata* (27 %, $p < 0.05$). The study has shown the differences in the ability to form biofilms among different *Candida*sp. and identity in the rate of detection among patients with and without blood system tumors.

Conclusion. The ability to form biofilms was various in different *Candida* sp. and predominated over *C. tropicalis* and *C. krusei.* The production of biofilms was detected with the same frequency in patients with tumors and without tumors of the blood system.

The study included 33 PV and ET patients receiving pegylated interferon therapy, of which 10 patients received previous treatment. The change of therapy was carried out due to intolerance or

insufficient effectiveness of the previous treatment. The comparison groups included 23 patients treated with hydroxyurea and 7 patients who received recombinant interferon. After 6 months of therapy, 43% of patients who were administered pegylated interferon alfa demonstrated complete clinical and hematological remission; 36% of them had partial clinical and hematological remission; the disease was stabilized in 21% of patients; none of the patients had disease progression. There was also a decrease in the JAK2 allele load. In the group of patients receiving pegylated interferon alpha, a statistically significant decrease in the median level of erythrocytes (p=0.0137), hemoglobin (p=0.0051), hematocrit (p=0.0051), platelets (p=0.001), leukocytes (p=0.002) was achieved after 12 months of therapy relative to baseline values. In the group treated with Hydrea, a statistically significant decrease in the JAK2 allele load (p=0.0066), RBC median (p=0.0003), Hb (p=0.0014), HCT (p=0.0031), PLT (p=0.000), and WBC (p=0.0005) were detected after 12 months of therapy in relation to the initial values. In the group of IFN, a statistically significant decrease in PLT (p=0.0156) was found after 12 months of therapy in relation to the initial values. When comparing the dependence of the effect of therapy on the variant of therapy used, no statistically significant differences were obtained (p= 0.2462 (Fisher's exact test). A correlation was revealed between the clinical and hematological response to therapy and the JAK2 allele load: the lower the allele load, the better the response to therapy. Moreover, this was observed in all the analyzed groups. The dynamics of histological changes was assessed in 7 patients in group 1: positive dynamics was noted in 2 patients.

100 patients with recurrent/refractory chronic lymphocytic leukemia (CLL) who obtained targeted monotherapy with Bruton's tyrosine kinase inhibitor (ibrutinib) were included into the study. An independent unfavorable prognostic effect of complex karyotype (5 and more chromosomal abnormalities) (HR: 4,27; 95% CI 1,5 - 12,1, p = 0,006) and 17p/TP53 deletion (HR: 6,18 (95% CI 1,8 - 20,3, p = 0,003) on progression-free survival was shown. The structure of 13q14 deletion and its prognostic value as the only disturbance found with the FISH method was studied using the material of 256 patients with CLL prior to FCR-like therapy courses. Various deletion options were found considering the size of the lost material, allele involvement and mechanism leading to genomic loss. Statistically significant differences in the total survival of patients with isolated deletion without and with the *Rb1*i nvolvement were obtained: 10-year total survival was 91.5% and 47.5% respectively, p=0.05. In 26 of 92 patients with CLL who underwent a standard cytogenetic study, the structure of unbalanced restructuring was detailed with molecular and cytogenetic mFISH (multicolour FISH) and mBAND (multicolour banding) methods. The two largest groups were identified: translocated chromosomal duplications without chromosomal partner deletion and unbalanced translocations with deletions. During analysis of marker chromosomes, additional unbalanced aberrations were detected. As a result, 3 patients were redistributed into a group of risk taking into account complex disturbances in the karyotype. The *CIITA/16p13.13* gene locus was studied in 37 patients with primary mediastinal (thymic) large B cell lymphoma with Sanger sequencing methods, FISH and standard cytogenetic study. Various somatic variations in the *CIITA* gene affecting its functionally important sites have been identified, which is of interest for further study of tumor biology and determining the relationship of *CIITA* disorders with the clinical course of the disease.

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

Implemented results of the project resulted in the development of new monocoatings in the form of powder and a kappa-carrageenan based sponge, bacterial cellulose, and coatings in the form of a sodium

alginate-based sponge. A composition of sodium alginate and zeolite, sodium alginate application with added ferric sulphate II and a combination of sodium alginate and iron oxide nanoparticles was developed (Russian patent No. 2739490). A new test system was offered to carry out a complex *in vitro* analysis of haemostatic activity of samples that have the form of a sponge. It was shown that sodium alginate coating can be used as a donor to ensure rapid delivery of therapeutic drugs to the injured area. The obtained *in vitro* results fully confirmed the *in vivo* results. Addition of thrombin and fibrin monomer effectively decreased a number of PLT and fibrinogen with significant activation of coagulation reactions *in vitro*. Addition of iron oxide nanoparticles to the sponge minimized but not excluded the coagulation shift. The selection of compounds with anticoagulant (AC)/anti-platelet (row 1) activities and hemocompatible compounds not leading to hemolysis and without AC/anti-platelet (row 2) activities was made. Row 1 included as follows: cellulose sulfates, cellulose aminodesoxybutyl, and sulfated organosolv lignin. Row 2 included aminodextrans, deoxycellulose, hydroxyethyl starch, cellulose conjugate with dibornol.

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

Reducing the dose of tyrosine kinase inhibitors (TKI) II in patients with chronic myeloid leukemia (CML) did not lead to a decrease in efficacy. However, to select an optimal dose of II generation TKI with maximum effectiveness and minimal intensity, patients with CML having the signs of available drug-induced toxicity or high risk of adverse events development while taking TKI should be assessed during prospective study.

High-dose melphalan therapy and subsequent auto-HSCT in patients with multiple myeloma (MM) with dialysis dependent renal insufficiency improved the anti-tumor response (71% for full remission, 29% for a very good full remission) and made it possible to achieve the minimal renal response in 14% of cases. Hemodialysis was stopped in patients with a minimal renal response. Observation continued, but there was no need in replacement renal therapy for 24-100 months. High-dose chemotherapy with subsequent transplantation of hematopoietic stem cells (auto-HSCT) is an effective and safe method of treatment of patients with MM and Grade III acute renal injury.

Modern protocols of combined immunosuppressive therapy (CIT) including antithymocyte globulin (ATG) and cyclosporine A, allow most patients with aplastic anemia (AA) to obtain a stable positive therapy response. The modern program of treatment of AA patients, including the use of both combined immunosuppressive therapy and hematopoietic stem cell transplantation at various stages of the course of the disease, can significantly improve the likelihood of long-term overall survival of patients and their quality of life.

The prospective study reflects the actual clinical practice of MM therapy in Russia. A high frequency of diagnosis of the late stages of the disease was noted. Induction therapy for the absolute majority of MM patients in Russia is carried out by bortezomib-containing regimens, the effectiveness of which is comparable with global data.

MRD-negative status following auto-HSCT was accompanied with high values of 4-year PFS irrespective of the presence or absence of lenalidomide-containing maintenance therapy (58% vs 46%, p=0,3). Supportive therapy administered to patients with MRD positive status following auto-HSCT produces an effect on PFS.

**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**

For the first time in the Russian Federation, a register of patients with Gaucher's disease (GD) was created, which included 320 adult patients (92% of the total population), which allows obtaining reliable information about the phenotypic characteristics of the disease and the dynamics of clinical and laboratory parameters in the process of pathogenetic therapy. The study of highly specific biomarkers (chemokine CCL18 and glucosylsphingosine) can be used as additional values in the individual program of complex assessment of Gaucher disease activity, especially among splenectomized patients The study of the parameters of the response to treatment is of great practical importance: patients who have achieved the goals of GD treatment can be transferred to a supportive regimen of enzyme replacement therapy in a timely manner.

Algorithms of diagnostics of hypo- and hypercoagulation conditions, new approaches and possibilities to administer therapies of hereditary coagulopathies, multiple schemes of anticoagulation therapy in patients with thrombotic complications and obstetrical failures were developed. They help control bleeding in patients with hypocoagulation changes and prevent recurrent thrombotic cases in patients with hypercoagulation disturbances. Individualized programs of hemostatic replacement therapy and prevention of complications in the postoperative period in patients with severe hemophilia A are being developed, which will improve the quality of life of patients and reduce the economic costs of healthcare for the treatment of complications in this group of patients.

**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 features of the course of recurrence of multiple myeloma (MM) after transplantation of autologous hematopoietic stem cells, comparison of the clinical course of recurrence of MM after auto-HSCT, depending on the timing of its development, were determined. Early immunochemical relapse was diagnosed in 20% of patients and developed in the period from 3.9 to 11.8 months (median 7.1 months). Late immunochemical relapse occurred in 80% of patients in the period from 12.6 to 66.7 months (median 29.2 months) ($p=0.00023$). 5-year total survival was 93.0% in the late relapse group and 58.2% in the early relapse group ($p=0.0039$). In the presence of bone plasmocytes at the onset of the disease, immunochemical relapse after autologous transplantation is significantly twice as likely to develop in the early stages (84.6% vs. 42.3%, $p = 0.01$). A significant prognostic factor is the response to the +100 day of auto-HSCT. In patients who have achieved complete remission, relapse develops later and proceeds like monoclonal gammopathies.

It was found that following allogenic transplantation, the lack of HLA-ligand in a patient for KIR donor significantly improves relapse-free survival (85% vs. 36%, $p=0.0131$) although there was no effect on the overall survival (68% vs. 54.6%, $p=0.077$). The increased graft-versus-leukemia reaction does not result in an increased probability of both acute (absence of ligand in 21% of cases and presence of ligands in 26% of cases, $p=0.6733$) and chronic (18% vs 10.5%, $p= 0.5777$) GVHD.

**Topic VIII: development of diagnostic sets of reagents for molecular and genetic detection of oncogenic transcripts for the purpose of early diagnosis and monitoring of minimal residual disease in acute leukemia**

As part of the project planned for 2020, an analysis of data published in open sources was carried out. As a result, 14 promising genetic markers – oncogenic chimeric transcripts determining the maximum number of cases of acute leukemia in adults were selected, namely: PICALM-MLLT10, PML-RARa, MLL-AF4, MLL-AF6, MLL-AF9, MLL-AF10, MLL-ENL, MLL-ELL, CBFB-MYH11, AML1-ETO, ETV6-RUNX1, E2A-PBX1, BCR (190 and 230)-ABL1. Moreover, the values of mRNA expression of the WT1, PRAME, EVI1, HMGA2 embryonal genes were selected as additional markers with a higher possibility of early diagnostics and monitoring of therapy of acute leukemia. A search for and theoretical development of design of oligonucleotide primers and probes, and their optimal combinations to ensure the multiplex real-time PCR were done. The standard samples of required nucleotide consequences were developed. The real-time PCR reactions were optimized, and the analytical sensitivity of methods was assessed. Preliminary studies to develop the methods of detection of the studied markers in the samples of dry spots of blood, and methods to develop original nano-magnet constructions at the stage of nucleic acid secretion were conducted. Selective comparative studies detecting chimeric transcripts within the samples were performed using the results obtained in independent laboratories. Preliminary studies of venous blood samples were performed in 55 patients with acute leukemia. The values of diagnostic sensitivity of the developed test systems were comparable to the literature data.