SUMMARY OF FINAL ANALYTICAL REPORT

Topic I: examination of molecular, genetic and immunomorphological basis of clonal blood disorders to improve diagnostics and find prognostic factors to make differential therapy optimal

1. It was found out that genetic abnormalities in groups CLL#1, CLL#6 and CLL#3 among patients with chronic lymphocytic leukemia (CLL) occur more frequently as compared to literature data. This could be partly due to the use of more sensitive methods. In addition, some of the patients in the study cohort had a relapse after the FCR regimen. It is also impossible to exclude population differences in the development of the disease.

The effectiveness of NGS technology (next generation sequencing) was shown to determine the mutational status of *IGHV* genes in difficult cases when Sanger sequencing was undescriptive. It was determined that in case of two displacements of *IGHV* family genes, *IGHV3*variants (68.7%) and *IGHV*-free variants are more common. Thus, the NGS method makes it possible to specify the diagnosis and administer proper treatment for a significant number of complex cases.

The method determining mutations in the C481 residue of *BTK* was improved based on the method of allele-specific polymerase chain reaction (PCR). A good correspondence of the results in determining the type of mutation and allele load with the NGS method was shown.

The first data on the spectrum of TP53 mutations in Russian patients with B-cell lymphomas have been obtained.

Inclusion of molecular and genetic factors of *BCL-2* rearrangement and *EZH2* mutations into the prognostic model in the presence of a follicular lymphoma (FL) increases its significance and allows patients to choose risk-adapted therapy options.

2. The protocol of a novel prospective clinical trial was created for patients with acute myeloid leukemia (AML) within the interregional scientific interaction of Russian hematology clinics. A prospective randomized controlled multi-center AML-21 trial will help to develop the unified modern approaches to diagnostics and treatment of patients with AML. Interaction of laboratory centers, academic staff and clinicians enables high-tech diagnostics and therapy of patients with AML in many Russian regions. The unified electronic database was developed to take into account the laboratory trial results in patients with AML.

Effectiveness of therapy using hypomethylating agents (azacitidine, decitabine) in patients with AML younger than 60 years and ATO+ATRA therapy in patients with acute promyelocytic leukemia (APL) were assessed.

Detection of minimal residual disease (MRD) is an important aspect of assessing effectiveness of the performed therapy and relapse monitoring. Methods with extremely high sensitivity are used for this purpose (10^{-4}-10^{-5}). However, the study of MRD in AML is not

standardized. The approaches proposed in different studies vary significantly. We suggested the options of MRD testing with multicolor flow cytometry demonstrating the used strategy of gating, described immunophenotype of healthy non-tumor hematopoietic cells and several options of MRD assessment. The panels of monoclonal antibodies used are also given with a description of their features.

The second option of MRD test includes analysis of targeted mutations within the healthy population with PCR. Improved protocol of *NPM1* analysis made it possible to expand the analytical possibilities of MRD detection with real-time PCR by 7%.

Optimization of the conditions of complex molecular genetic analysis in AML patients made it possible to narrow the intermediate risk group by 5% due to the introduction of the *RUNX1* gene into routine practice.

3. Sensitivity of E. faecium and E. faecalis to antibiotics was studied during various periods of the study (2002-2009 and 2010-2017). It was noted that the share of vancomycin-resistant strains among E. faecium increased from 8.3 to 23.4 % ($p = 0.0001$) during the second study period (2010-2017). Two linezolid-resistant strains were isolated. All vancomycin- and linezolid-resistant E. faecium were still daptomycin- and tigecycline-resistant. All strains of E. faecalis were sensitive to tigecycline, linezolid and teicoplanin. Only one strain with moderate resistance to vancomycin was found. E. faecalis had a steadily high sensitivity to ampicillin (97.7 and 97.6% respectively).

In sequencing of 83 vancomycin-resistance E. faecium (VR-E. faecium) it was determined that they belonged to 22 sequence types (ST) with three predominant sequence types (ST17, ST78 и ST80) that included 54.3% of studied VR-E. faecium. All VR-E. faecium belonged to the same genetic line, a clonal CC17 complex, commonly found in hospitals around the world.

Sensitivity of planktonic forms and Candida spp (8 C. krusei, 7 C. tropicalis, 7 C. albicans, 5 C. parapsilosis) biofilms to antifungal agents was examined. Sensitivity of Candida spp. planktonic forms to anidulafungin, capsofungin, and fluconazole was 100%, 85.2% and 66.7% respectively. MIC90 of amphotericin B was ≤ 1 mg/ml. Both tested echinocandins were in vitro active against Candida biofilms with MIC80 of anidulafungin being lower as compared to capsofungin. All Candida biofilms were fluconazole (MIC80 >1024 mg/ml) and amphotericin B (MIC80 was ranged within 4-16 mg/ml) resistant.

Thus, higher rates of antibiotic resistance were determined for E. faecium, which was manifested in an increase in the proportion of vancomycin-resistant E. faecium and the appearance of linezolid-resistant strains. The clonal composition of VR-E. faecium underwent changes. However, high risk clones (ST17 and ST78) remained common and can be considered endemic to

Russia. Anidulafungin had a higher in vitro activity against Candida spp. biofilms; it was lower in capsofungin and totally lacking in amphotericin B and fluconazole.

4. FL, DLBCL and HGBL-related EMAST, MSI, LOH were examined. LOH, EMAST, and their combination made it possible to get a group of patients with FL and HGBL who had an unfavorable course of the disease. No adverse effect of LOH and EMAST was produced on the survival of patients with DLBCL. Mononucleotide repeat aberrations that were against MSI-H have no independent prognostic value.

In DLBCL with signs of unfavorable prognosis in accordance with the international prognostic models, R-CHOP therapy results were found to be unsatisfactory with overall 5-year survival rate being not higher than 35%.

Different options of DLBCL therapy pharmacoeconomic effectiveness (PEE) were analyzed. It is noted that R-NHL-BFM-90 is more effective than R-DA-EPOCH with a difference in the prevalence of grades 3-4 hematological toxicity but without lethal outcomes.

WBC dynamics was analyzed following R(G)-DHAP immune therapy in patients with non-Hodgkin lymphomas. It was found that the activation period of independent granulocytopoiesis does not depend on the stimulation of G-CSF and the total dose of growth factor and corresponds to an average of 10-11 days of break after the end of the course of chemotherapy. This makes it possible to have an optimal prevention mode of G-CSF when the cytotoxic effect is over. The duration of neutropenia can be minimized with delayed development in case of early hyperleukocytosis. Moreover, the phenomenon displays the fundamental features of G-CSF interaction with bone marrow progenitor substrate and granulocytopoiesis chronology as a general property of hematogenesis.

5. Within 8 years, a sample of 108 patients with primary myelofibrosis was formed; an early (prefibrous) stage was diagnosed in 35 (32.4%) patients. The median age was 48 years (21-65 years); male/female ratio was 1:2.9 (9:26). It was diagnosed based on the criteria of WHO developed in 2017. Splenomegaly was found in 26 (74%) cases. The median rate of PLT and WBC was 650 $x10^9$/L (313 $x10^9$/L to 3472 $x10^9$/L), and 9.04 $x10^9$/L (4.5 $x10^9$/L to 17.6 $x10^9$/L). The molecular characterization was as follows: 40% for JAK2V617F, 46% for CALR, 3% for MPL and 11% for triple-negative disorder. The history of thrombosis was recorded for 4 (11%) patients: 2 cases of ACVA and 2 cases of thrombosis in the system of portal vessels. Concurrent disorders were diagnosed in 31 (88.5%) of cases. In the first line of therapy, patients were administered hydroxycarbamide (34%), interferon alpha (43%), and observation only (23%).

Data of 57 patients with unclassifiable myeloproliferative neoplasm were analyzed. The median of age was 45 years (22-83 y.o.), the male/female ratio was 1:1.3 (25: 32). 28 (49%) patients developed splenomegaly with the median of PLT and WBC being 672 $x10^9$/L (39 $x10^9$/L

to 2158 x10^9/L) and 8 x10^9/L (2, 7 x10^9/L to 62,9 x10^9/L). Molecular properties: 75% for JAK2V617F, 16% for CALR, triple negative disorder in 9% of cases. During the diagnosis, 17 (30%) patients had a history of thrombosis: ACVA (5), myocardial infarction (4), portal vein thromboses (8), haemorrhoidal veins (1), celiac trunk (1), renal artery (1), and deep veins of the shin (1). 4 patients developed 2 thrombotic events each. The first therapy line included hydroxycarbamide (40%), interferon alpha (11%), and symptomatic therapy (49%). In the first stage of therapy, splenectomy was performed in 3 cases.

Previously prepared histochemical preparations that had undergone Masson and Gomori trichrome staining were used to perform the morphological analysis of the grade of stromal bone marrow reticulin fibrosis and grade of bone marrow osteosclerosis in previously selected patients using the semi-qualitative method of assessment and placing encoded results (0-3 corresponds to four grades of myelofibrosis and osteosclerosis) into an already created table with the clinical profile of patients.

6. The plasmacytoma genetic profile in multiple myeloma (MM) was examined. 10 patients with MM complicated by plasmacytoma were included in a retrospective study (7 females, 3 males) with the median of age of 53.5 years (34 to 62 y.o.). Paired samples of tumor/control DNA were isolated from 10 patients for subsequent STR-profiling. All 10 patients were reported to have loss of heterozygosity with different allele load resulting in either deletion/qualitatively neutral loss of heterozygosity (HL) or duplication of one of two alleles. Thus, the work took the first step towards the molecular genetic study of MM and collected the first data on the loss of heterozygosity in the genome of plasmocytes. There was a tendency to a greater number of loci with HL in the substrate with plasmocytes from the MM relapse group, compared with plasmocytes detected at the onset of the disease. When analyzing the data, we noted a common HL on the following chromosomes: 1 (1q42), 6(6q14), 7 (7q21.11), 13 (13q31.1), 21 (21q21.1).

Histological and IHC values of plasmocytoma were detected in 21 patients with MM. The rate of NSD2 protein and D1 cycline expression within the bone plasmocytoma substrate of patients with MM was 33.4% and 28,6% respectively. Comparison of chromosomal aberrations detected within the bone marrow using the FISH method and expression of oncogene products within the bone plasmocytoma substrate can be indicative of clonal heterogeneity of MM and activation of various tumor clones in bone plasmocytomas.

76 patients with MM (43 males and 33 females) with the median of age being 58 years (35 to 82) were included into a prospective trial from February 2019 to July 2021. The level of MAGE-C1 expression was found in CD138+ enriched bone marrow samples of all patients using real-time PCR. The results of the study suggest that the increased expression of the MAGE-C1 gene is

associated with high proliferative activity of the tumor and the volume of tumor mass and can be considered as an additional marker reflecting tumor activity in MM.

7. Leukemic clone kinetics was assessed after withdrawal of tyrosine kinase inhibitors (TKI) in patients with chronic myeloid leukemia (CML) with a preserved deep molecular response (MR) after TKI withdrawal including analysis of remote monitoring outcomes. It was noted that the MRD rate varied in 66% patients with CML and deep MR after TKI withdrawal. In 74% of cases MR precedes the molecular relapse such as loss of a great molecular response (GMR). MR loss at less than 3 months term after TKI withdrawal was considered as the most unfavourable event resulting in the development of molecular relapse. During the analysis of remote observation results of treatment-free remission (TFR) is was noted that 2 years after TKI withdrawal the possibility of GMR loss was 51% whereas 5 years after TKI withdrawal only single molecular relapses were observed.

The 20-year overall survival(s) of CML patients who started imatinib therapy as part of the GIPAP charity program was evaluated. In patients with early and late chronic phase (CP), 20-year TS was 67% and 58% respectively. Leukemia-related deaths dominated in the structure of mortality reasons. The rate of CML deaths in patients with early and late CP was 8% and 25% of patients respectively (p=0.008).

Concentrations of TKI in patients with CML and deep MR included into the study of TKI dose de-escalation were examined. No significant differences in nilotinib concentrations were reported during the therapy with a standard dosage of 400 mg and reduced dosage of 200 mg. This can be taken into account when making decisions about TKI dose de-escalation in patients with deep MR.

The results obtained are important for the development of the principles of de-escalation of treatment of TKI and management of patients with treatment free remission (TFR), as well as for the construction of an optimal strategy for the treatment of patients with CML. These trends are planned to be continued in further work to solve practical problems and optimize targeted therapy in patients with CML.

Topic II: examining the possibility of using hematopoietic allotypic antigens as targets of hemoblastosis cellular therapy

In the framework of studying the possibility of using hematopoietic allotypic antigens as targets of hemoblastosis cellular therapy, a panel of 24 allotypic antigens origintaing from genes expressed by myeloid cells as a potential target of hemoblastosis immune therapy was formed. It was experimentally confirmed that synthetic peptides were associated with predicted HLA-A*02:01 and HLA-B*07:02 alleles. In silico DNA cassette was constructed with a sequence of

candidate peptides to be express in antigen-presenting cells and to detect the processed antigen. A panel of blasts was selected in patients with primary/recurrent AML/ALL; it was phenotyped by the presence of HLA-A*02:01 and HLA-B*07:02 alleles. DNA was prepared for typing of alleles of the studied polymorphisms. Whole exome sequencing of 3 donors was performed to detect a genotype using the studied polymorphisms.

In addition, the applicability of alloantigens as targets for cellular immunotherapy of hemoblastoses was also studied. A method is being developed for the prevention or therapy of recurrent hemoblastosis after transplantation of allogeneic stem cells using donor lymphocytes specific to the patient's alloantigens encoded by genes predominantly expressed in hematopoietic tissue.

Topic III: implementation of molecular-genetic and biological mechanisms in the human body in normal and in diseases of the blood system

1. In patients with diseases of the blood system with multiple transfusions before allo-HSCT, the true group affiliation was determined by erythrocyte systems in order to search for markers for subsequent monitoring of HSC engraftment and prediction of possible immunological complications. Prior to transplantation of hematopoietic stem cells (HSC), 98 patients had difficulties in assessing the serological results for detection of group affiliation: 79 patients with posttransfusion chemirism and 19 patients with a low expression of erythrocyte antigens. Genotyping was done in 49 patients with posttransfusion chemirism. The genotype was specified in 14 patients with a low expression of erythrocyte antigens. ABO typing was done in 32 patients: 25 with posttransfusion chemirism and erythrocyte agglutination with coloclones (5-98%) and 7 with a low expression of antigens A (6 patients) and B (1 patient). Patients with 50-80% red cell agglutination and a-A1 colyclone had ABO*O1A2 genotype (group A2), whereas himerism was observed in the presence of donor A1 red cells. ABO*O1A1 (group A1) was identified in a patient with 50% red cell agglutination with a-A1 colyclone; the presence of donor A2 red cells was the reason for himerism. A patient with 50% red cell agglutination with a-A and a-B colyclones had ABO*B1B1 genotype.

21 patients had typing to detect Rhesus system genes. Posttransfusion chimerism by RhD antigen (70%) was detected in one patient only. She had a rare genotype(RHD+ RHCE*ccee). The presence of RHCE*C gene was confirmed in 10 patients with 50-100% of red cell agglutinated anti-C colyclone. Only 1 patient with 30% anti-C agglutinated red cells had homozygosity by RHCE*c gene. 5 patients with 20-95% anti-C agglutinated red cells had homozygosity by RHCE*C. 7 patients with 10-60% anti-E agglutinated red cells had confirmed homozygosity by RHCE*e. 20 patients had typing to detect MNS system genes. Patients with 50-90% a-M colyclone

agglutinated red cells and 50-80% a-N colyclone agglutinated red cells had NN genotype. Patients with 90-100% a-M colyclone agglutinated red cells and 50-80% a-N colyclone agglutinated red cells had MM genotype. Patients with 100% a-M colyclone agglutinated red cells and 50-80% a-N colyclone agglutinated red cells had the MN genotype. Patients with 100% a-M colyclone agglutinated red cells and 70-80% a-N colyclone agglutinated red cells had MM genotype. 4 patients had typing to detect Kell system genes. Patient with 50% a-K and a-k colyclone agglutinated red cells was homozygous for the KEL*01.01 gene. 3 patients with 80-97% a-K colyclone agglutinated red cells had the KEL*01.01 и KEL*02 genes.

Genotype was double checked in 15 donors. Reasons: no blood group-related data were available upon arrival of HSC cryopreserved cells, and it was impossible to perform a test due to RBC lysis (in 4 donors); specify genotype to find out informative markers of HSC engraftment monitoring (1 patient for MNS); weak antigen expression (10 donors and 14 patients had confirmed O1A2, B1B1, RHD weak type 1, RHD weak type 2, RHD weak type 3, RHCE*Cw genes).

Math models of vascular thrombogenesis with intense hemodynamics were validated as illustrated by arteriovenous fistulas. Methods of numerical stimulation were used to study systemic complications of ischemic insults. A technical solution to include the method correcting personalized thrombotic risk indicators based on topological and spectral analysis of electocardiographic data into a cloud app was developed. A model of activated thrombogenesis in the intense blood flow was developed considering VWF molecule proteolysis. Parametric diagrams of thrombocyte activation were created in 'length of the unwound von Willerbrand factor' and 'time of being unwound' axes and in 'the number of von Willerbrand factors' and 'time of being unwound' axes.

2. PCR systems to analyze the primary structure of all functionally important areas of the ADAMTS13 (29 exons), ITGA2B (30 exons) and ITGB3 (15 exons) genes were developed. Defects in the ADAMTS13 gene of 7 patients (Upshaw-Schulman syndrome (USS)) could be detected using a complete mutation analysis carried out for 18 patients diagnosed with thrombotic thrombocytopenic purpura (TTP). Mutations in the ADAMTS13 gene correlated with the lack of antibodies specific to ADAMTS13 protein. High-titer antibodies (41-44 BU) were detected in one patient only who was a heterozygous microinsertion c.4143insA carrier. Two new missense mutations (p.Trp387Ser and p.Arg116Pro) that have never previously occurred in the global population were found.

Pathogenic variants of p.Arg1060Trp and c.4143insA turned out to be major for the domestic population, at least one of them was present in each patient. This makes it possible to develop cost-effective systems of effective differential molecular and genetic express analysis that

allows to differentiate between the inherited and acquired forms of TTP within the shortest term (during a day). This is extremely important since fundamentally different therapeutic approaches are used for their treatment. All functionally important areas of the ITGA2B and ITGB3 genes were analyzed in 4 patients diagnosed with Glanzmann thrombasthenia (GT). The analysis made it possible to detect a new pathogenic variant of the ITGA2B gene (c.1810 insC), which had previously never occurred in the global population. A targeted NGS panel was developed to analyze gene mutations (40 genes) associated with various hereditary defects of thrombocytopoesis. In accordance with the results of the first year, a panel of monoclonal antibodies was developed and processed to detect the composition of small populations of T cells. The first group of samples taken from 7 healthy donors were processed as well to establish the reference values of T cell quantitative characteristics such as naive T cells, central memory cells, effector memory cells and terminal effector cells. Complex technique of gating and isolation of 52 various subpopulation of T cells was developed as well. The planned work has been completed in full, but it is planned to continue collecting samples from different donors.

Topic IV: 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

This work evaluated the reconstitution of the T-cell link of the immune system in patients after transplantation of allogeneic hematopoietic stem cells. The effect of different regimens of GVHD prevention on the pool of T cells was demonstrated. The basic mechanism of immunosupression following transplantation (depletion of naive T cells) was displayed. In the study, the prognostic value of minimal residual disease prior to the transplantation of allogenic hematopoietic stem cells in patients with acute leukemia was studied. It was shown the MRD produced a negative effect on the relapse values following transplantation, which significantly decreases the total survival of these patients. A large clinical group was used to assess the results of autologous HSCT in patients with multiple myeloma and dialysis dependent renal insufficiency and patients with AL-amyloidosis. There was no transplantation-related mortality. With a median follow-up of 53 months, 5-year progression-free survival was 59% with total survival being 93%. Effectiveness of transplantation of allogenic hematopoietic stem cells in patients with mantle cell lymphoma (MCL) and mutations in the TP53 gene was studied. It was shown that transplantation is effective in this case.

Topic V: 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 disease (GD) was created, which included 340 adult patients (> 90% of the total population), which made it possible to obtain 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 the response parameters to treatment makes it possible to transfer patients who have achieved the goals of GD treatment to a supportive regimen of enzyme replacement therapy in a timely manner.

In 30% of cases, patients with autoimmune hemolytic anemia (AIHA) are shown to be resistant to a standard immunosuppressive therapy. The least effective outcomes are observed in cold forms of AIHA. In 45-85% of patients with resistant AIHA, hemolysis can be stopped with rituximab, i.e. monoclonal antibodies to CD20 B-cells. The preliminary analysis shows the effectiveness of reduced doses of rituximab in the treatment of patients with resistant forms of AIHA and the expediency of using rituximab as the first line of AIHA therapy with cold agglutinins and a mixed type of autoantibodies. Clinical recommendations for the diagnosis and treatment of paroxysmal nocturnal hemoglobinuria (PNH) and acute porphyria (AP) have been developed and approved (2021) by the Ministry of Health of the Russian Federation.

Algorithms have been developed for the diagnosis of hypo- and hypercoagulation conditions, new approaches and opportunities for prescribing therapy for hereditary coagulopathies, various anticoagulation therapy schemes in patients with thrombotic complications and obstetric failures, allowing to control bleeding in a group of patients with hypocoagulation changes and prevent repeated thrombotic cases in patients with hypercoagulation disorders.

Individualized programs of hemostatic replacement therapy and prevention of complications in the postoperative period in patients with severe hemophilia A can improve the quality of life of patients and reduce the economic costs of healthcare for the treatment of complications in this group of patients. Combined haemostatic therapy of factor VIII and tranexamic acid is recommended in large orthopedic surgeries to reduce post-operative blood loss and decrease post-operative complications. Constant infusion of VIII factor should be used in patients with a short factor VIII half-life who underwent large orthopedic intervention in the early post-operative period.

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

A prospective study "Early induction therapy and monitoring" in patients with CML showed that the frequency of achieving a deep molecular response is high, which will further increase the proportion of patients in remission without treatment.

The analysis of the effectiveness of the R-B and R-CHOP courses in patients with FL showed that the R-B scheme is more effective in FL 1st and 2nd, and R-CHOP - in 3 cytological types. The volume of the tumor (especially the presence of bulky), the FLIPI index and the morphology of FL are the key criteria for choosing therapy.

When nelarabine is administered in adults with refractory/recurrent acute T-cell lymphoblastic leukemia/lymphoma, the obtained data are not so optimistic. 3 of 10 patients had a recurrence in less than 1 year after allo-HSCT. In 2 patients with total remission, the observational period after allo-HSCT was 9 and 11 months. However, the inclusion of nelarabine in chemotherapy regimens for these forms of T-ALL can improve the results of chemotherapy.

The results of the program therapy of acute myeloid leukemia demonstrated that for patients with FR after the 1st course, but with MRD-positive status, it is necessary to perform allo- HSCT. Moreover, allo-HSCT should be performed as early as possible.

Targeted therapy with ruxolitinib in patients with polycythaemia vera allows effective control of symptoms, decreases the risk of thrombotic complications and improves the TS values.

Administration of vemurafenib, BRAFV600E inhibitor, to patients with hairy-cell leukosis prior to administration of standard medications for patients with deep neutropenia/agranulocytosis as preliminary therapy prior to the standard course with cladribine decreased the rate of infectious complications and increased the rate of PR.

The creation of an Observational Department in the event of epidemiologically unfavorable situations was a rational decision that made it possible to reduce the risk of the spread of infectious diseases, establish the correct diagnosis in time for patients, provide medical assistance in the development of life-threatening conditions, continue therapy, including high-dose chemotherapy, and optimize the activities of a medical organization without the risk of large economic loss.

Longer duration of treatment and depth of MR (≤ MR4.5) in patients with CML, the likelihood of maintaining remission without treatment increases before the withdrawal of TKI. Our research has shown that survival without molecular relapse does not significantly increase with the use of TKI2 in the first line compared with imatinib. Nevertheless, TKI2 therapy as the first line allows halving the duration of treatment necessary to achieve comparable survival rates without molecular relapse compared to imatinib.

100% molecular remission and recurrence-free survival were achieved owing to therapy of patients with APL based on the differentiated approach depending on the group of risk and with addition of cytostatic drugs to patients from a high-risk group only. Unsuccessful therapy (early

mortality and death in the remission phase) in high-risk patients is explained by severe condition of these patients when APL was diagnosed.

A study on the use of brentuximab vedotin in the Russian Federation has shown that the drug is effective and well tolerated for the treatment of late stages of CD30+LPZ. The use of brentuximab vedotin in an unfavorable cohort of pretreated patients with cutaneous T-cell lymphomas showed rather promising outcomes with the possibility of achieving a large percentage of the overall response with acceptable toxicity.

Outcomes proving effectiveness and cost-efficiency of R-DA-EPOCH and R-mNHL-BFM-90 chemotherapy ± auto-HSCT in patients with DLBCL have shown that R-mNHL-BFM-90 is the most effective and cost-efficient strategy with the least therapy expenses. The scheme allows to achieve CR and prevent expenses associated with the use of the second and subsequent lines of anti-tumor therapies. As a result, total expenses are significantly decreased.

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

Prototypes of diagnostic testing systems have been developed based on multiplex PCR technology to perform additional testing of patients with suspected acute leukemia (Leukemia Screen), to specify the molecular and genetic profile (Leukemia-Diff) and control the effectiveness of AML therapy (Leukemia-Monitor-M) and ALL (Leukemia-Monitor-L). The developed sets can be used to detect mRNA expression of five ocogenes (WT1, PRAME, EVI-1, HMGA2, BAALC) and 14 chimeric transcripts (PML-RARa, PICALM-MLLT10, MLL-AF4, MLL-AF6, MLL-AF9, MLL-AF10, MLL-ENL, MLL-ELL, CBFB-MYH11, AML1-ETO, ETV6-RUNX1, E2A-PBX1, SIL-TAL, BCR-ABL1(p190, p210, p230) during the same working day. Standard samples that control whether the analysis is done correctly have been developed. A well pronounced heterogeneity of mRNA oncogenic expression was shown and the hypothesis of specific features in the ratio of oncogenic mRNA expression in leukemogenesis was formulated. The algorithm that interprets the Leukemia-Screen test results considering the detected ratio of RNA markers is helpful as it ensures a high level of diagnostic effectiveness with 96% sensitivity and 71% specificity. Based on the performed testing, a patent application for the invention entitled 'Method of diagnostics of acute leukemia and related sets' was developed; 'Technical conditions' of the set production were formulated as well.

Topic VIII: influence of architectural and structural changes of new polymer compounds on their hemocompatibility and provision of hemostatic reactions in in vivo and in vitro experiments

The study of architectural and structural changes in local coatings, namely: determining the shape of coatings (film, sponge, gel plate), their *in vitro* effect on the formation of a primary thrombus in direct contact with blood, the dependence of the hemostatic activity of coatings on the introduction of micro- and nanoparticles of iron oxides, analysis of physical and chemical properties, the study of various technological processes for obtaining coatings, — allowed us to propose to further develop the production of new local hemostatic coatings based on chitosan and bacterial cellulose. The data were analyzed, and it was shown that chitosan-based coatings are active only in case of low bleeding (up to 1.25 g.min). When micro- and nanoparticles of iron oxide were added to the coatings, hemostatic activity was significantly increased irrespective of the bleeding rate. The absorptive power and density of some samples were increased as well. *In vitro* results showed better generation of thrombin and consumption of thrombocytes (189.50 to 141.20 $x10^9$/L) when the sponge contacts the blood. The structural changes in the coating with bacterial cellulose did not result in decreased haemostatic activity (73.55-87.84%) but increased the porosity of samples, which requires further investigation. Work devoted to the choice of a manufacturing process of these coatings continues.

Sulfated galactolucomannan having a polydispersion rate of 1.43; sulfated galactolucomannan having a polydispersion rate of 1.5; galactomannan obtained by sulfate crystallization with sulfamic acid and urea having a polydispersion rate of 2.75, and galactomannan obtained by sulfate crystallization with chlorosulfonic acid in 1.4-dioxane do not result in aggregation of human thrombocytes and produce no effect on *in vitro* human red blood cell hemolysis. In intravenous infusion of sulfate to experimental animals (117 alla U/kg) its hemorrhagic activity is 20 times less as compared with that of non-fractioned heparin (same dose). Layer-wise modification of polyurethane plates with quaternized chitosan and unfractioned heparin results in high thromboresistance.