Almazov Federal
North-West Medical
Research Centre

INSTITUTE OF HEMATOLOGY

of Almazov Federal North-West Medical Research Centre

www.almazovcentre.ru

THE HISTORY OF THE CENTRE

Centre's foundation

1980

Almazov Federal North-West Medical Research Centre was founded in 1980 as an Institute of Cardiology.

Scientific and clinic departments

2002

In 2002, the institute was named after its founder Vladimir Almazov.

New Buildings

2006

In 2006, the structure was improved- new high-tech: inpatient and outpatient clinic with 480 beds for 360 visits per day.

Federal Perinatal Centre

2010

In 2010, the first in the North- West region Federal Perinatal Centre was opened.

2015

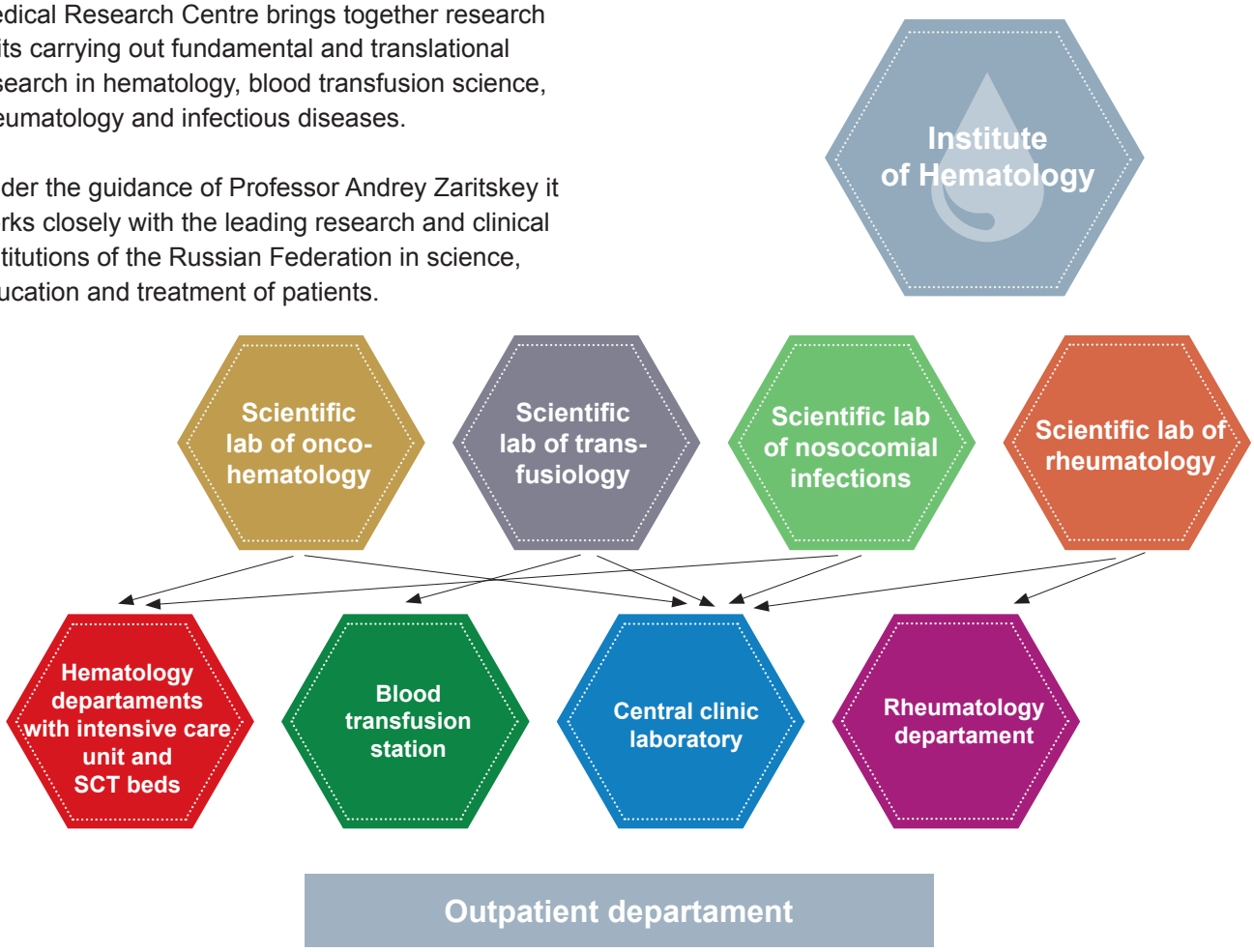
Rehabilitation Complex 2 was opened.

In 2015, Polenov Neurosurgical Institute was affiliated to the Centre.

INSTITUTE OF HEMATOLOGY
of Almazov Federal North-West Medical Research Centre

Institute of Hematology of Federal Almazov North-West Medical Research Centre brings together research units carrying out fundamental and translational research in hematology, blood transfusion science, rheumatology and infectious diseases.

Under the guidance of Professor Andrey Zaritskey it works closely with the leading research and clinical institutions of the Russian Federation in science, education and treatment of patients.

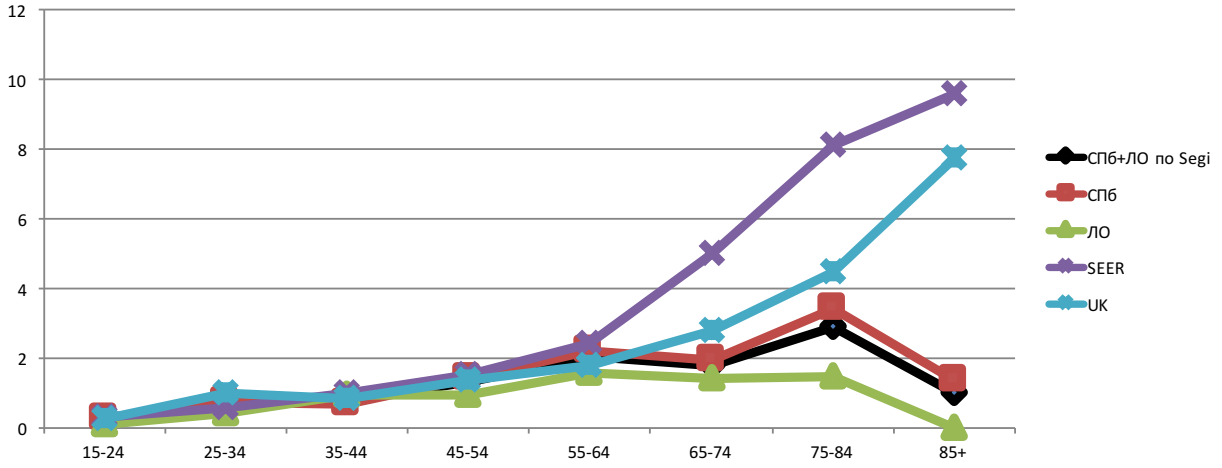


CML

One of the main directions of the scientific activity of the Institute of Hematology in the field of hematology is to study epidemiology and pathogenesis of myeloproliferative diseases, the influence of genetic aberrations on their course and response to target therapy. Some of the research programs were supported and performed in close collaboration with European LeukemiaNet. Within the project «The European Treatment Outcome Study (EUTOS) for CML» of European LeukemiaNet the epidemiology of chronic myelogenous leukemia (CML) in Saint Petersburg and the Leningrad region was studied for the first time. The incidence of CML, standardized by age and sex (Fig. 1) was evaluated. The data were nearly the same as in other registries in all age groups except in patients older than 70 years. In this group the CML incidence was much lower and this needs further explanation. We revealed that the prevalence of CML increased almost two fold during 5 years of observation in these regions of Russia (Fig. 2). It is certainly related to the efficacy of tyrosine kinase inhibitors (TKI). Data of superiority of TKIs over previous available interferon therapy are apparent.

During nearly 15 years we studied the efficacy and tolerability of TKIs in patients with CML and searched for the mechanisms of resistance to this drugs in both first and the subsequent lines. The influence of different clinical and laboratory factors, including molecular markers, on early and long-term results of CML therapy, has been evaluated. It was revealed that the depth (or deepness) of the response to previous therapy is the most relevant prognostic factors besides BCR-ABL mutations. The results of this research leads to therapy individualization and will help to choose of optimal treatment strategy, including allogeneic hematopoietic stem cells transplantation (allo-SCT).

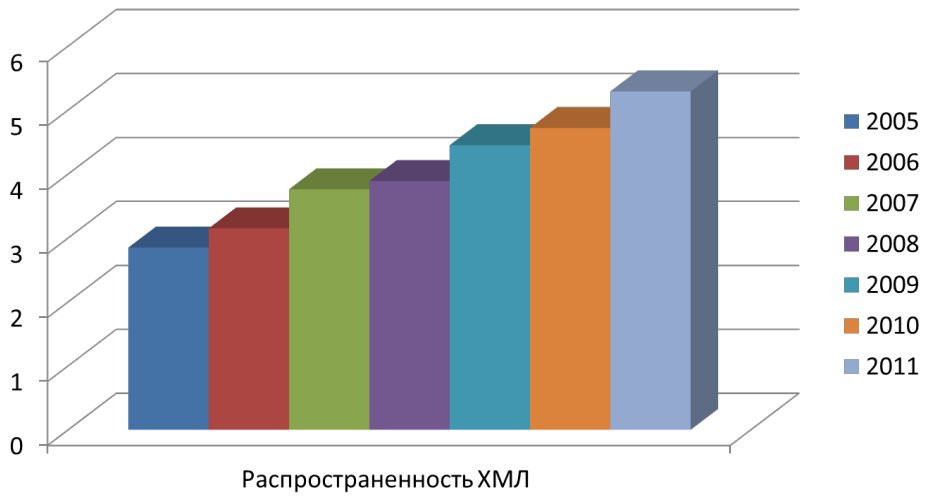
Fig. 1. Age standardized CML incidence in SPB and Len reg (by Segi) and in other registries



Age groups	SPB+Len reg	SPB	Len reg	SEER (1)	UK(2)
15-24	0,23	0,27	0,12	0,3	0,28
25-34	0,67	0,75	0,42	0,6	1,01
35-44	0,78	0,71	0,97	1	0,82
45-54	1,3	1,41	0,97	1,5	1,35
55-64	2,05	2,22	1,6	2,4	1,78
65-74	1,8	1,95	1,4	5	2,8
75-84	2,9	3,4	1,45	8,1	4,49
85+	1,01	1,37	0	9,6	7,74

1 – http://SEER.cancer.gov
2 – Phekoo et.al. Haematologica; 2006; 91(10)-data from South Themes

Fig. 2. CML prevalence in St.Petersburg and Leningrad region

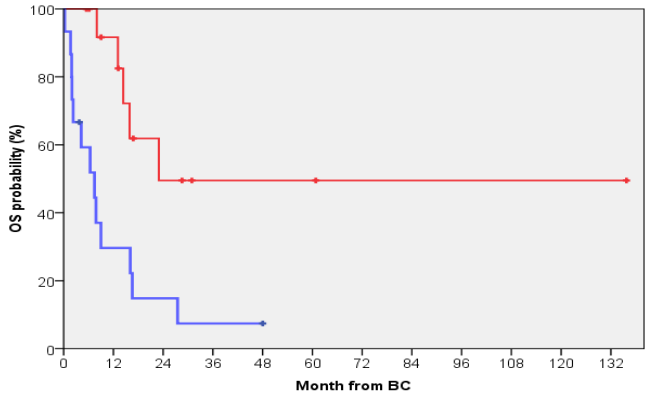


Parameter	At the end of							Rise
	2005	2006	2007	2008	2009	2010	2011	2006-2011
Rate of alive pts	187	207	247	255	292	310	347	85,6%
CML prevalence	2,85	3,15	3,76	3,89	4,45	4,72	5,29	x 1,85

We also optimized approaches to the therapy of patients in advanced phase of CML. Therapeutic protocols to bring patients with blast crisis of CML to alloHSCT are in being developed. A haploidentical allo-SCT has been introduced and is being actively used as it allows shortening time to alloHSCT, which

is extremely important in the treatment of patients with advanced phases of CML where TKIs and chemotherapy have only temporary effect. Due to the active pre-transplant management and usage of different donor type, the survival rate in this extremely unfavorable group of patients increased and now exceeds 50% (Fig. 3).

Fig. 3. Successful use of alloSCT in adults in patients with CML BP



Therapy with TKIs in CML is usually life-long and thus their safety profile is extremely important. Data of the effect of these drugs on the cardiovascular system are being constantly accumulated. To study the role of different TKIs, we begun the project to study the prevalence of metabolic changes and preclinical atherosclerosis in patients with CML who do not have a history of cardiovascular events. Over 100 patients were examined at different TKIs. Preliminary data indicate an increase in the incidence of various factors, including primarily hyperlipidemia, in patients receiving TKIs (in particular nilotinib) compared with the general population comparable by gender and age. However, at this stage, an increase in the incidence of preclinical atherosclerosis was not detected in this group of patients (patients are mainly with low and middle group on the SCORE scale). This work is ongoing. Several clinical trials are being held in the centre with novel drugs for CML treatment.

The staff of the hematology institution actively shares own experience, gained in the course of clinical and scientific activities in the field of studying CML with the colleagues during the round tables and conferences in Russia and also in the post-Soviet countries (Uzbekistan, Belarus, Georgia, Kazakhstan, Ukraine). The Institute of Hematology is the only center in Russia participating in the ERSAP program of the iCMLf organization, whose goal is to spread the excellence of knowledge in CML in countries with emerging economies. According to this program, hematologists from Uzbekistan, the Republic of Belarus, Kazakhstan, Armenia, passed training courses in the Institute of Hematology and hematology departments of the Center.

Fig. 4. Education activity the staff of Hematology institution

СОВЕТЫ СПЕЦИАЛИСТА

Школа для пациентов с хроническим миелолейкозом

22 ноября 2012 года в Центре им. В.А. Алмазова прошла очередная школа для пациентов с хроническим миелолейкозом. На этот раз основной темой обсуждения стало появление новых препаратов-дженериков. О преимуществах новых способов лечения нам расскажет бессменная ведущая школы ведущий сотрудник НИЛ онкогематологии к.м.н. Елза Галактионовна Ломаиа.

– Елза Галактионовна, расскажите, что такое дженерики и зачем они нужны?

– Современная фармакология неуклонно развивается в направлении роста эффективности и безопасности препаратов. Разработка высокотехнологичных лекарств требуют огромных средств как на этапе доклинических, так и во время клинических исследований. Требования к регистрации оригинальных препаратов очень жесткие и компании-разработчики обязаны доказать их эффективность и безопасность во многих межнаро-

ты аналогичны по действующему веществу, но могут отличаться друг от друга по вспомогательным компонентам. В исследованиях на небольшом количестве людей (как правило, это здоровые добровольцы) сравнивают их биоэквивалентность через определенные промежутки времени путем изучения концентрации в крови, а также пути выведения из организма дженерика и оригинала. Это позволяет в разы снизить стоимость воспроизведенных препаратов. Снижение цен на эти препараты может сделать их доступными для

обычную соду или мел. Подделка – это не лекарство! Копии лекарства – это те препараты, которые произведены с нарушениями патента. Это может быть идентичный по названию препарат или произведенный в период действующего патента. Дженерики в этом сравнении имеют очевидное отличие. В них активное вещество абсолютно идентично основному веществу оригинального лекарства.

– О каких дженериках идет речь в случае лечения хронического миелолейкоза?

Ведущий научный сотрудник НИЛ онкогематологии к.мн. Елза Галактионовна Ломаиа

Автономная некоммерческая организация по оказанию помощи больным онкологическими и онко-гематологическими заболеваниями

содействие

Благодарственное письмо

Ломаиа Елзе Галактионовне

к.м.н., ведущему научному сотруднику Института гематологии ФГКЭ им.В.А.Алмазова

В Международный день поддержки пациентов с ХМЛ выражаем искреннюю благодарность и признательность за ответственное отношение к порученному делу и высокий профессионализм в работе.

Благодаря Вашему непосредственному участию и программе «7-ля нозологий» пациенты с хроническим миелоидным лейкозом имеют возможность получать дорогостоящее лечение согласно мировым стандартам, что позволяет не только спасти людям жизнь, но и на неограниченное время сохраняет их трудоспособность и социальную активность, оставляя полезными для страны, общества и семьи.

Искренне желаем Вам крепкого здоровья, стабильности и процветания, счастья и благополучия, верных соратников и успехов во всех делах.

Директор Матвеева Л.Ф.

International CML Awareness Day

Schools for CML patients are being held at regular basis. They helps to improve knowledge about the disease, increase adherence to therapy and improve the results of treatment in CML patients. The staff of the Institute of Hematology is actively involved in the project named «The right to live» initiated by the All-Russian social organization of patients with oncohematologic diseases. Within the framework of this program, patient schools, as well as seminars for doctors, are being organized all over Russia (Fig. 4).

Experts from hematology institution in CML are active members of Russian CML study groups and are involved in the development of National recommendations for the diagnosis and treatment of patients with CML (publication of the recommendation update is currently in progress).

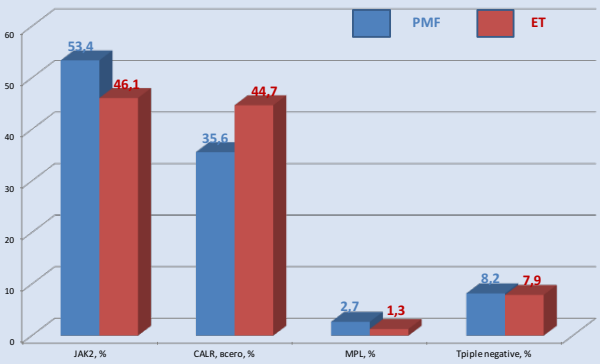
The Institute of Hematology of North-West Medical Research Centre is an active member of the European LeukemiaNet (ELN) (www.leukemia-net.org) since 2007. Since then, Federal North-West Medical Research Centre staff regularly attends events held by ELN: annual symposium on acute and chronic leukemia in Mannheim (Germany), as well as separate meetings of the working groups on the CML which are regularly held in Heidelberg (Germany) and in the framework of the world's major symposia of European and American Association of Hematology. In the course of these activities several reports on the work results were made: in July 2010 in Heidelberg, Director of the Institute of Hematology Andrey Zaritskey presented the results of a prospective study of patients in the Northwest region of Russia, and in October 2013 at the symposium "ELN Frontiers Meeting" in Vienna (Austria) the Institute presented a poster report on long-term results of targeted therapy of CML in the Northwest region of Russia.

Ph-negative myeloid neoplasms

There is also a project to study clinical and molecular predictors of disease course and therapy response in patients with «Classical» Ph-negative myeloid neoplasms. We have been estimating the incidence of different mutations in this group of patients (Fig. 5) and confirmed that patients with different molecular profiles have distinct clinical course, especially regarding the risk of thrombosis. Overall, patients with JAK2 V617F mutation are more at risk of thrombotic events than patients with CALR and MPL mutations or so called «triple negative» patients.

To evaluate the mechanisms of transformation from essential thrombocythemia (ET) and polycythemia vera (PV) to myelofibrosis (MF) the diagnostic role of WT1 gene expression was studied. All patients with secondary or primary MF but not PV or ET had elevated expression of WT1. Now we are studying whether WT1 may be prognostic marker for acute leukemia transformation in these groups of patients.

Fig. 5. The incidence of JAK2V617F, CALR, MPL mutations in patients with ET and PMF



Chronic lymphocytic leukemia is the most common type of leukemia in the adult population of Russian Federation. The Institute if Hematology has long lasting interest in both fundamental and clinical aspects of this disease, being one of the few CLL-specialized centers in the country. Fundamental research is focused mainly on the discovery and validation of new disease markers and evaluation of clinically relevant mechanisms of leukemic cells functioning in bone marrow and lymph node microenvironment. A specialized program of state of the art medical care for CLL patients has been established, providing a free, comprehensive and up-to-date evaluation and treatment for all referred cases. The list of routinely implemented laboratory analyses includes flow cytometry, cytogenetics, FISH, screening for autoimmune complications, evaluation of IGHV-mutation status, estimation of minimal residual disease by flow cytometry according to standardized protocol and screening for TP53 and NOTCH1 mutations with confirmatory DNA sequencing.

The gold standard for the treatment of CLL in the last ten years has been the FCR regimen. Since 2002, the staff of the Institute has been collecting a database of patients who received fludarabine-containing regimens. Now 90 patients have a follow-up exceeding 15 years. With such long follow-up, the advantage of the FCR regimen has been shown, especially in the cohort of young patients, who have preserved kidney function. Kidney pathology was the most significant comorbidity which affected life expectancy in our patients. The favorable prognostic significance of the mutated variant of IGH-genes has been shown (fig. 6.1, 6.2, 6.3, 6.4)).

In recent years, a new highly effective and low-toxic cytostatic drug, bendamustine, has appeared in the therapeutic arsenal for CLL. Since February 2012, with the support of the pharmaceutical company Astellas, the Institute initiated a large-scale observational clinical study BEN-001-NORMA (clinicaltrials.gov id NCT02110394) on the basis of 37 specialized

Fig. 6.1. Event-free survival in FC and FCR subgroups

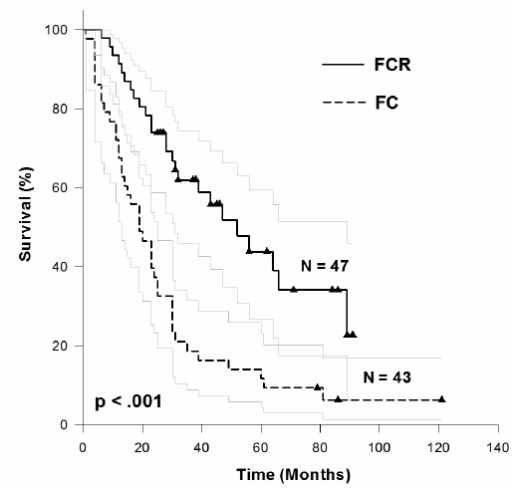


Fig. 6.2. Overall survival in FC and FCR subgroups

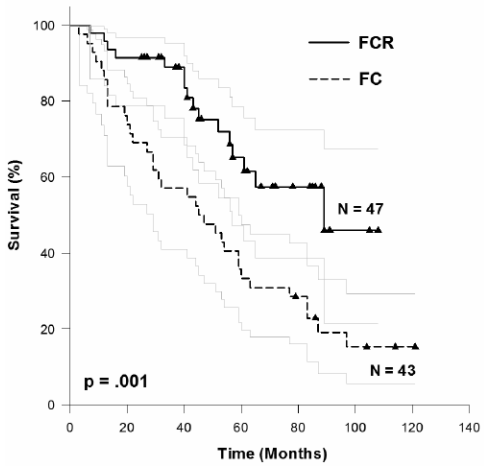


Fig. 6.3. Overall survival according to calculated creatinine clearance on FCR regimen (n = 90)

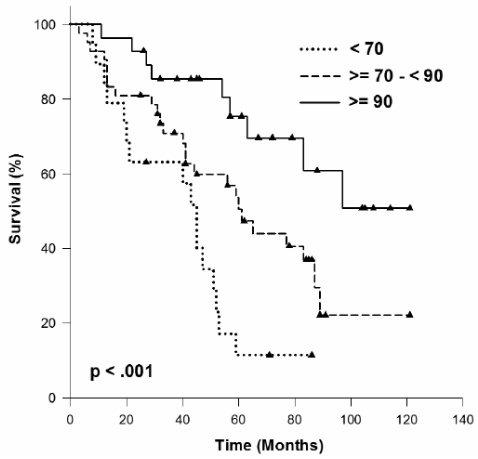
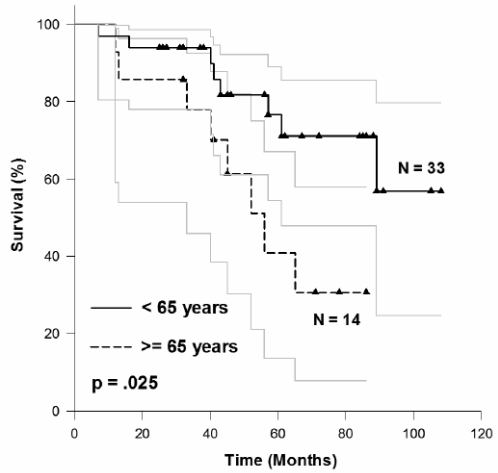


Fig. 6.4. Overall survival according to age in FCR subgroup



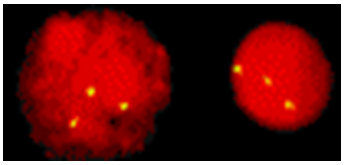
hematological centers located in 26 cities of Russia. In this study, the effectiveness and safety of bendamustine + rituximab regimen was assessed in previously untreated patients with progressive CLL. BEN-001-NORMA was the first multicenter clinical study of such magnitude in Russia. (Fig. 7) Within the framework of the laboratories of the Institute of Hematology, such studies as determination of the mutational status of IGHV-genes, assessment of minimal residual disease by flow cytometry and cytogenetic analysis were carried out. The study included 190 patients, which now continue on a follow-up. Overall response rate was 92% with 74% complete remissions. Twenty-two unfavorable events occurred during the median follow-up period of 26 months, 15 patients died. Three year progression free survival was 87%. (fig. 8, 9) Eradication of MRD (determined in the bone marrow) was achieved in 30%. (fig. 10) The main indicator of effectiveness, which correlated with progression-free survival, was precisely the eradication of minimal residual disease. (fig. 11). This turned out to be important even in a subgroup of patients with non-mutated IGH-gene variants. (fig. 12) On the other hand, the mutational status has retained its prognostic significance both in the general cohort and in the group of patients with MRD negativity after treatment (fig. 13).

At present CLL patients from different cities of Russia are being treated with intracellular signaling inhibitors in the Institute of hematology. The maximum period of observation for patients receiving ibrutinib is currently 3 years. Before starting therapy, patients are subjected

Fig. 7. BEN-001 NORMA trial

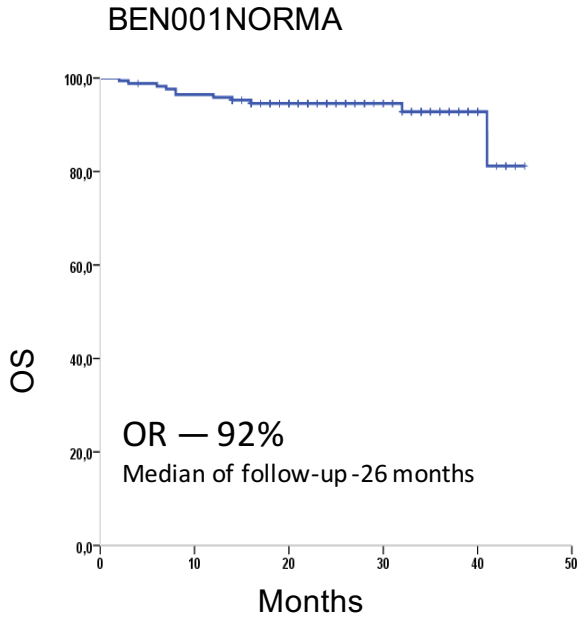
Research methods:

- Immunophenotyping by flow cytometry method
- Determinaion of mutational status of VH–genes
- MRD evaluation by flow cytometry method
- Cytogenetics and FISH
- Detecting of TP53 mutation by FASAY method
- Sequencing of NOTCH1-gene



clinicaltrials.gov NCT 02110394

Fig. 8. Overall response and 3-year progression-free survival



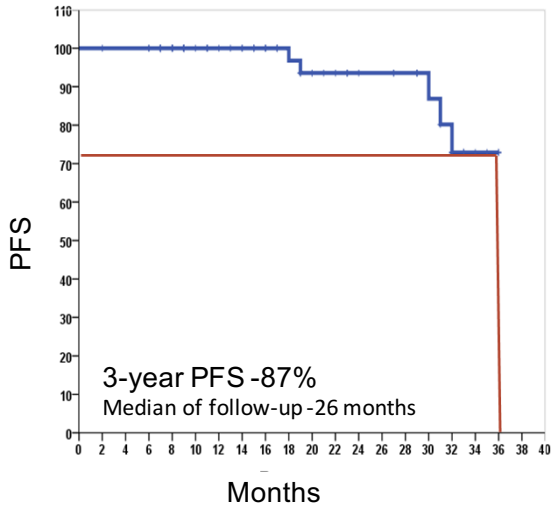


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Fig. 9. Overall response after 3 and 6 cycles of BR

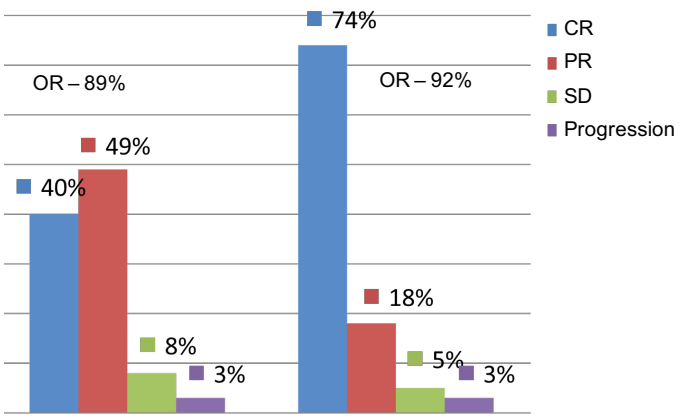

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Fig. 10. Minimal Residual Disease (MRD)

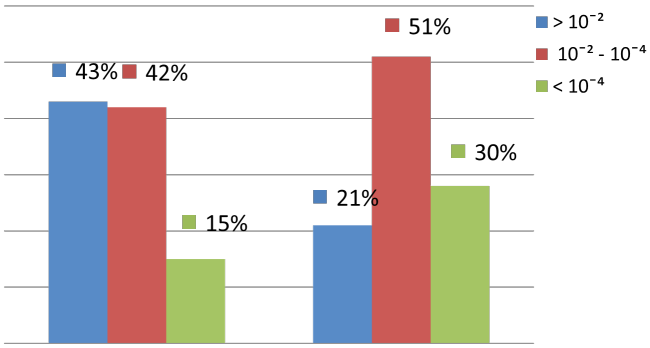

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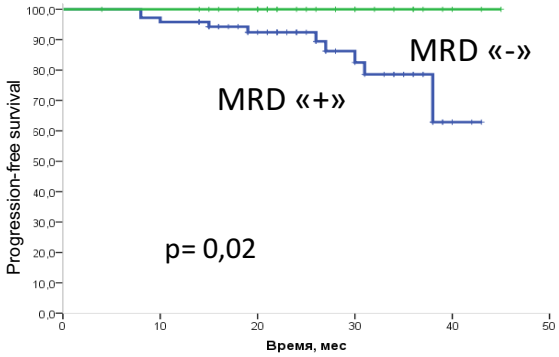
Fig. 11. MRD-negativity predicts better progresson-free survival



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to molecular studies followed by disease response monitoring. The complications of ibrutinib therapy and ways for their prophylaxis are being studied. The big problem now is the situation when patients have signs of progression of the disease while they are receiving ibrutinib. In this regard, the Institute initiated an early access program for venetoclax, which will include patients from all Russian cities who have progression on Btk inhibitors.

Fig. 12. Progression-free survival of IgHV-unmut patients is better if MRD-negativity achieved

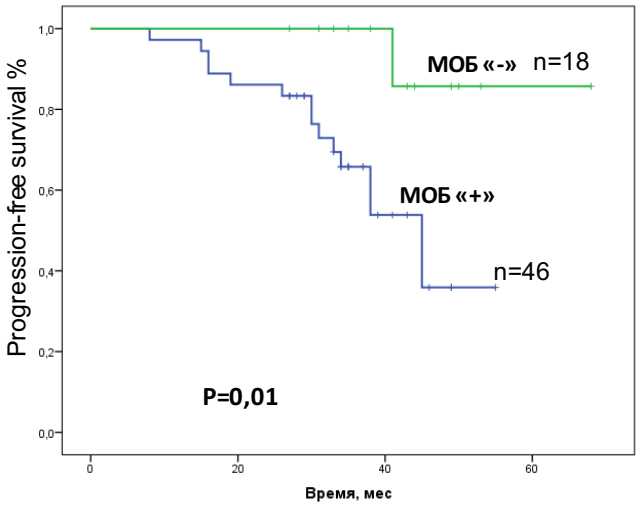

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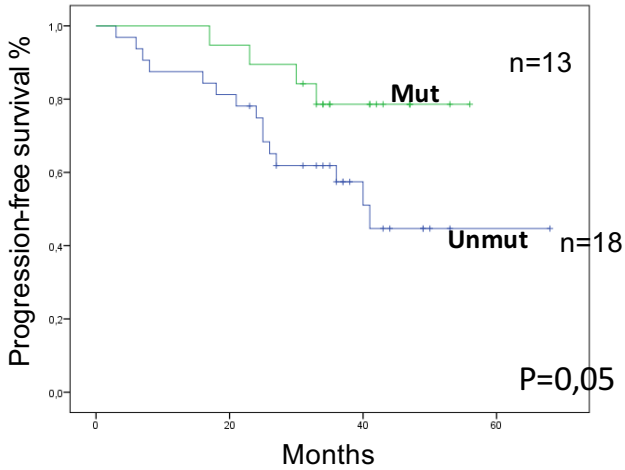
Fig. 13. Progression-free survival of MRD-negative patients: IgVH mut vs IgVH unmut



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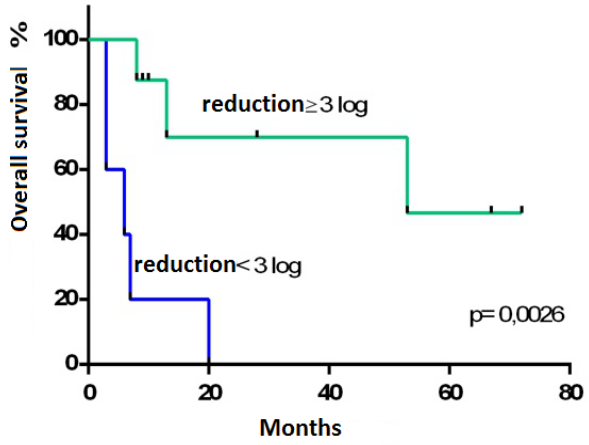
AML

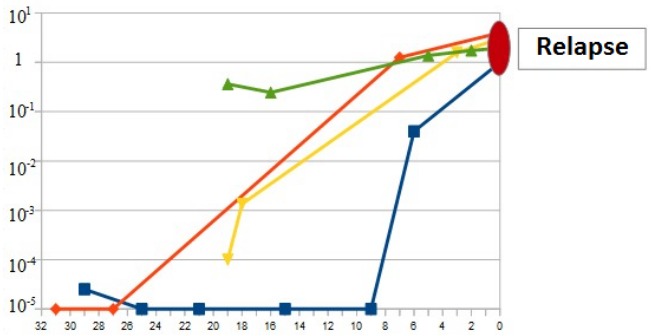
Intensive chemotherapy regimens for both AML de novo and AML relapses in the hematology department are being used. There were 135 patients with AML diagnosis during 2009 -2017 with AML de novo and relapse. The priority line is development and realization of risk-adjusted AML therapy. Therapy is based on molecular, cytogenetic, clinical and laboratory alterations present at AML onset and relapse. There is the possibility in our department to estimate different molecular markers for monitoring of remission and MRD.

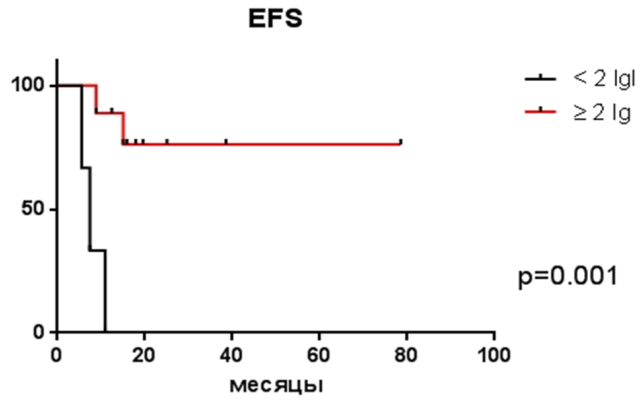
The current approach for treatment of acute myeloblastic leukemia includes the achievement of maximum tumor reduction and, therefore, eradication of a leukemic clone. AML with NPM1 mutation occurs in 30% of all AML cases and has favorable prognosis except the cases of combination of NPM1 and FLT3 ITD mutation. But relapses also can occur in NPM1 AML. It was observed that the frequency of relapses differs in some NPM1 subgroups. It is an important task to predict sensitivity of leukemia clone to therapy and one way for this might be the minimal residual disease (MRD) estimation (fig. 14).

■ Fig. 14.



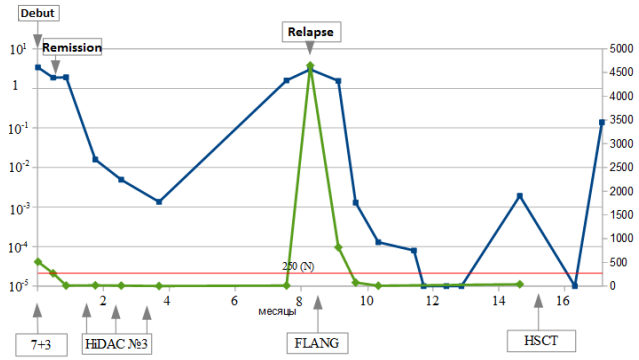
■ Fig. 15.1.



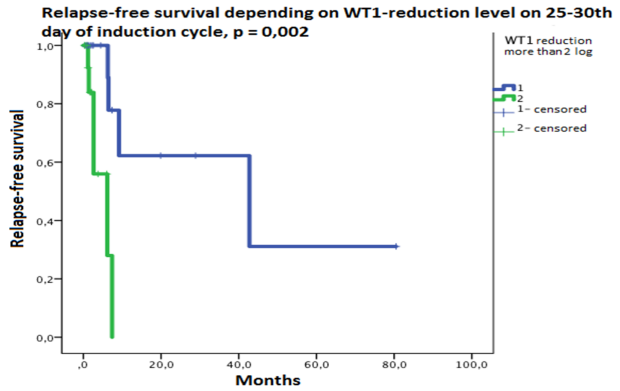


The incidence of relapses in a group with a decreased RUNX1-RUNX1T1 expression level of >2 log is 75% as compared to patients with a less significant reduction of the transcript level (with the relapse incidence equal to 0%) (p = 0.05). The increase of the RUNX1-RUNX1T1 level against the background of

■ Fig. 15.2.



■ Fig. 16.



bone marrow remission by more than 1 log coincided with a bone marrow relapse within 5–18 weeks. In addition, long-term persistence of a certain transcript level after the completion of a program therapy without relapse is possible (fig. 15.1, 15.2).

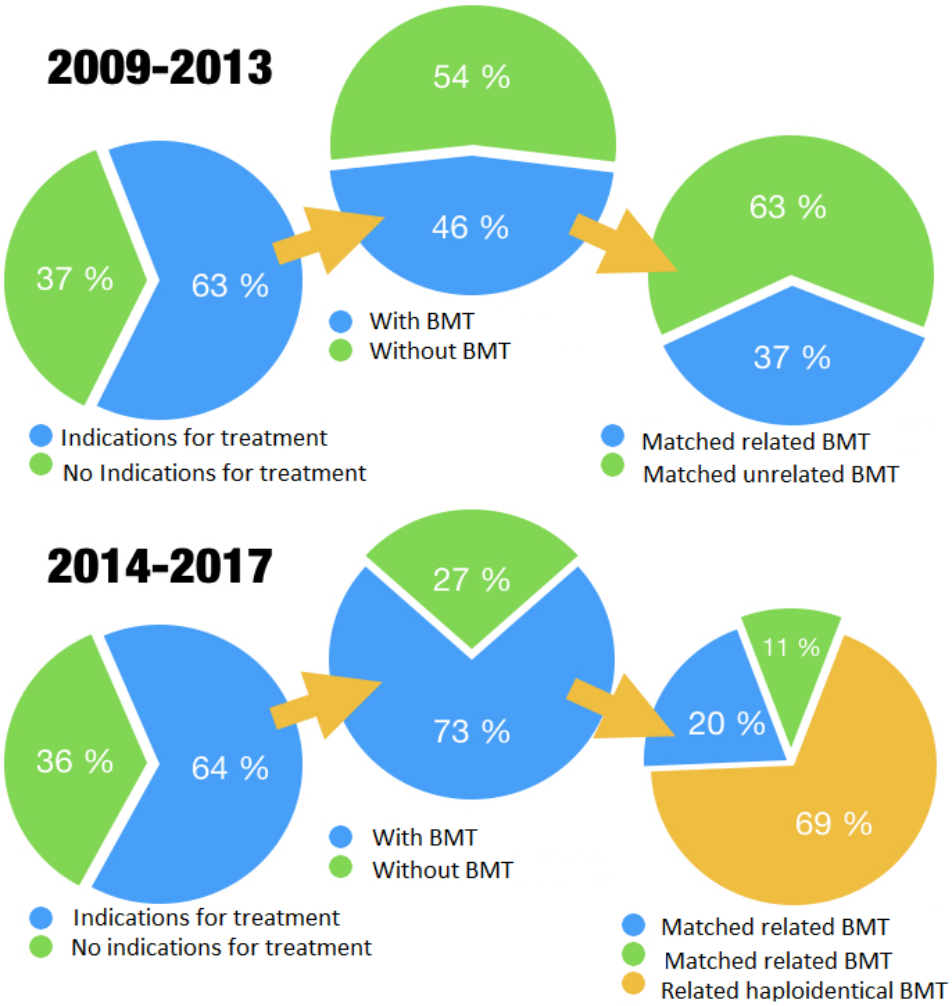
WT1 detection in bone marrow and peripheral blood are comparable in all cases of WT1 positive leukemia. WT1 level expression was also valuable for monitoring of second remission during the search and activation of unrelated donor. Specific markers appeared to be more sensitive than WT1 (fig. 16).

For AML relapses we use the effective chemotherapeutic regimen «FLAG». In our experience this regimen has good tolerance and acceptable toxicity in patients older than 60 years if they have good comorbidity status.

Hematopoietic stem cell transplantation

Bone marrow transplantation is carried out in Almazov Centre since 2009 – autologous, and since 2010 – allogeneic. Haploidentical related bone marrow transplants are performed in the center since 2014 (Fig. 17, 18). Our center has a membership in the European Bone Marrow Transplantation group (EBMT): temporary since December 2011 and a constant from April 2012. It has assigned the centre number CIC 925. To date, more than 400 transplantations have been carried out (multiple myeloma, lymphomas, Hodgkin lymphoma, acute myeloblastic leukemia, acute lymphoblastic leukemia,

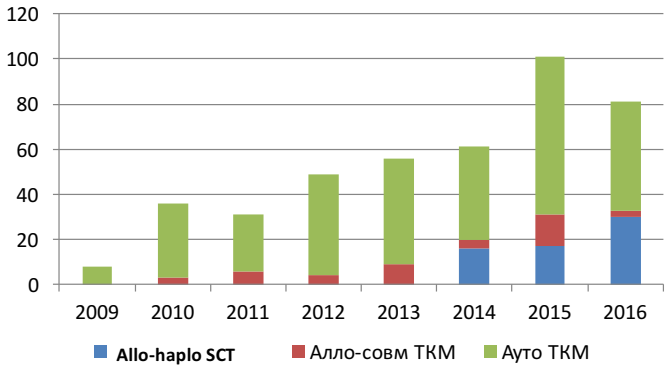
Fig. 17.



chronic myelogenous leukemia, chronic lymphocytic leukemia, myelofibrosis) and autoimmune (systemic scleroderma, systemic lupus erythematosus) should be noted. The center prepares patients for transplantation, including salvage therapy, examination and carrying out of BMT, as well as management in post-implantation period, observation and treatment of complications of BMT (GVHD, etc.).

In Almazov center we are eager to develop and apply new therapies, including cellular products. We are able to carry out all stages of preclinical: cellular and animal studies, and clinical trials of the new drugs. Currently, we are working on establishment of therapeutic products based on donor-derived mesenchymal stromal cells and anti-oncogenic lymphocytes.

Fig. 18. Transplantation activity in Almazov Federal Centre (2009–2016)

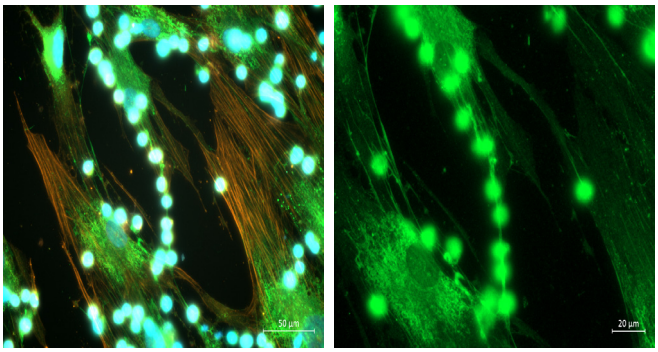


Translational research

The main topic of interest of the laboratory of molecular hematology is to study mechanisms of hematopoiesis and its alterations associated with malignant transformation. We believe that it is important to study hematopoietic stem cells in the context of complex signals that surround hematopoietic stem cells forming so called stem cells niche. One of the examples of our work is the study of mesenchymal bone marrow

stromal cells obtained from CML patients. We found that MSC's from patients with prolonged CML course, but not MSC from primary CML patients, on top of other, have a substantial senescence when cultured in vitro. This could be due to the course of the disease, or because of chronic intake of TKI's (Fig. 19).

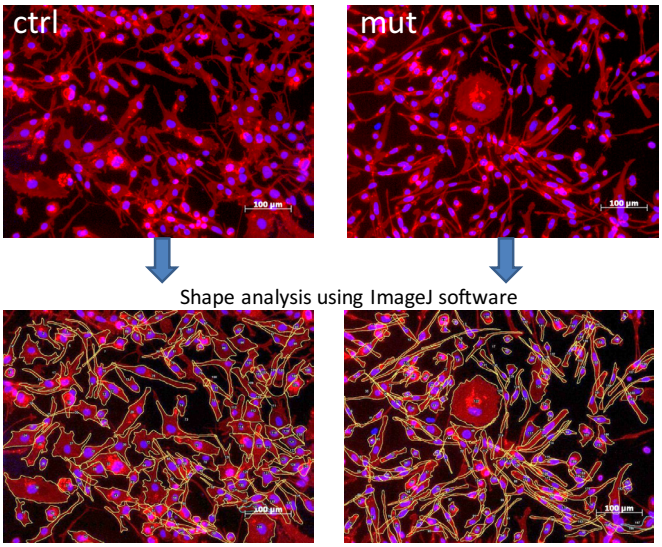
Fig. 19. In mixed culture BM-MSC/ fibrocytes are attached to the collagen fibers

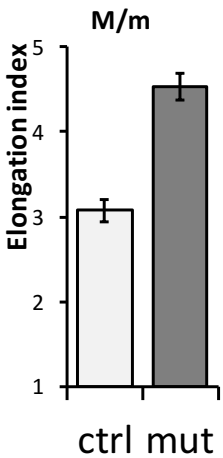


Green – Col1
Red – alpha-SMA
Blue – DAPI

Col1 – green

Fig. 20. Monocytic leukemia cells THP-1 with JAK2 V617F (mut) contains more elongated cells that unmodified (ctrl) after phorbol ester treatment

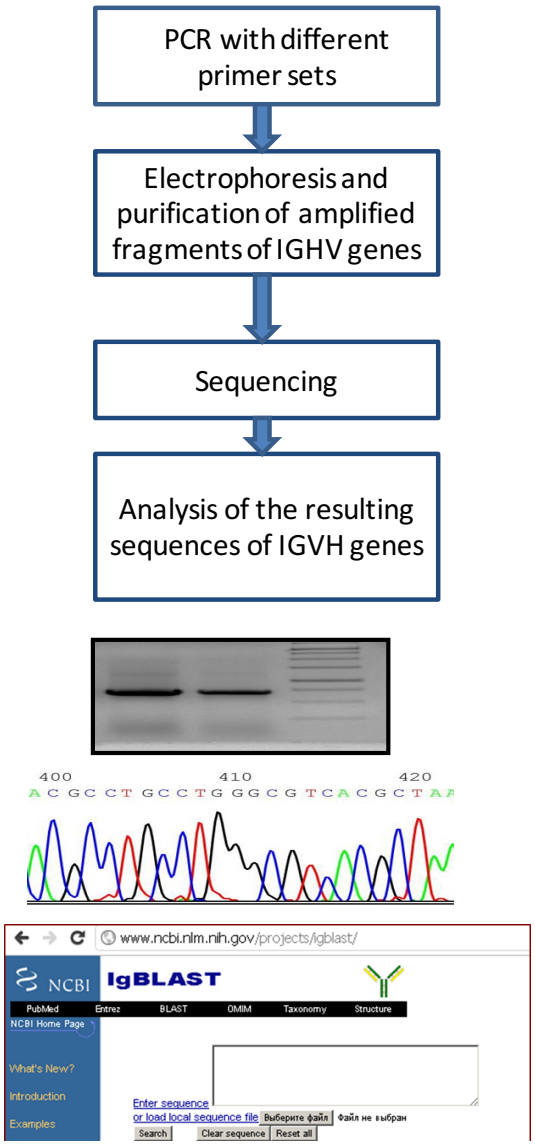




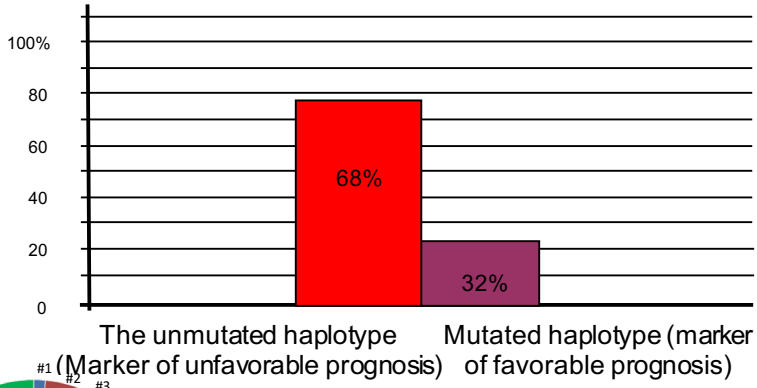
Mechanism of pathogenesis of bone marrow fibrosis is one of the key questions of modern hematology. The main paradigm of myelofibrosis is shifting from absolute role of megakaryocytes as orchestrator of the pathological process to more complex picture, including other myeloid cells, mainly of monocytic origin. In the Institute of Hematology we study how the oncogenic mutation JAK2 V617F affects monocytes, including their ability to differentiate into fibrocytes. On the patient side we study the interplay between molecular characteristics e.g. mutational status with myelofibrosis progression and complications, including thrombosis (Fig. 20).

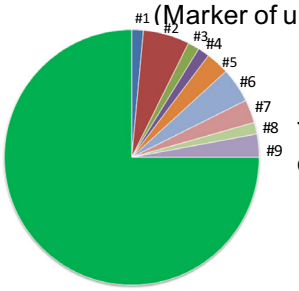
We are developing field of genetic markers of CLL. Our lab acquired certificates from ERIC for TP53 and IGVH mutation status analysis. Also we study mutation profile of CLL patients (Fig. 21.1, 21.2) who received

Fig. 21.1. Determination of the mutational status of the genes of the variable region of the heavy chain of immunoglobulins



■ **Fig. 21.2. Determination of the mutational status of the genes of epy variable region of the heavy chain of immunoglobulins**

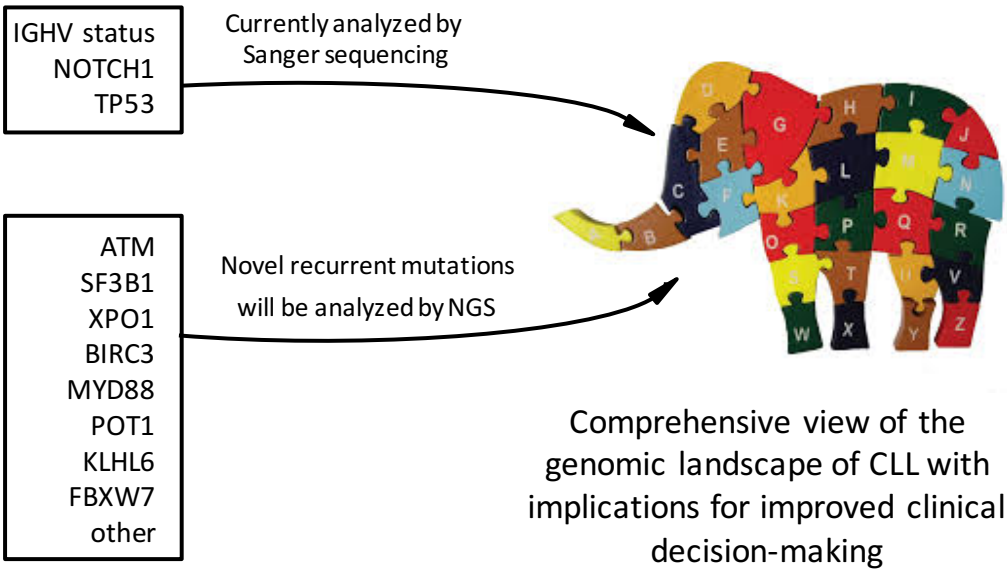




The analysis of features of stereotypy in the structure of the obtained sequences :

Subset 2 IGHV3-21, CDR3 9 aa DANGMDV

■ **Fig. 21.3. Targeted Next-Generation Sequencing in CLL**

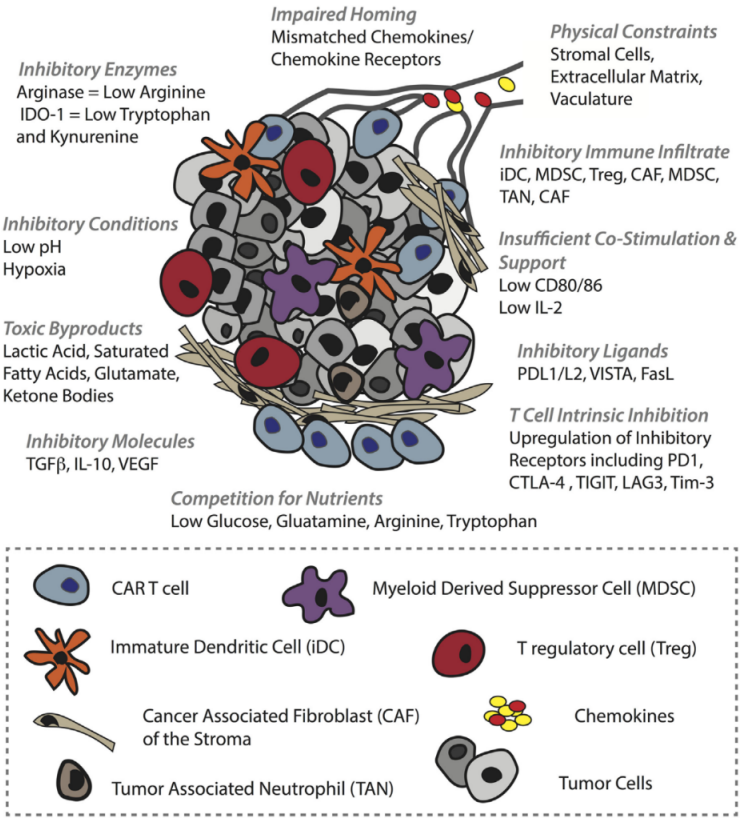


BR regiment on Illumina NGS platform, using custom panel, developed in the Institute of Hematology. (Fig. 21.3). This will help to develop optimized recommendation using genetic data.

CRISPR project is dedicated to target suppression of genes expression through an epigenome modification. The technology provides a genetic checkpoint-immunosuppressors correction inside hematopoietic stem cells ex vivo using chimeric dCas9 protein, guideRNA specific for an gene and viral vector delivery system. This cutting edge technology does not have analogs and has potential ability to eradicate nearly any cancer by immune system.

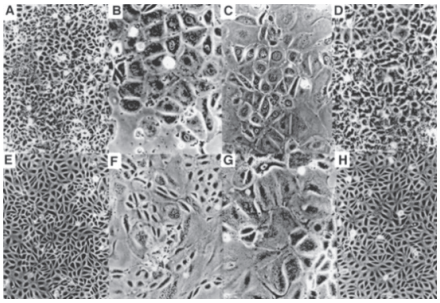
The aim of the CAR-T project is to produce universal antitumor T-lymphocytes expressing chimeric antigene receptor (CAR) (allogeneic CAR-T). This includes T-cells separation, genome modification of the T-cells, security and effectiveness tests of the product. There is no equivalent technologies in Russian Federation and this project is able to reduce the backlog in the field of cellular immunotherapy (Fig. 22.1, 22.2).

■ **Fig. 22.1. Tumor immunosuppressive mechanisms and the modified CAR-T, resistant to this immunosuppression**

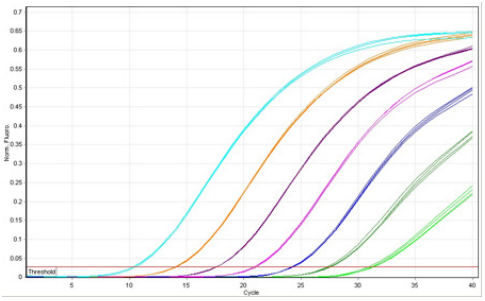


1. **RAID-peptide, upregulating T-cell response**
2. **shRNA_LAG3, shRNA_CTLA4 and shRNA_PD1 downregulating LAG3, CTLA4 and PD1 expression resulting in increased antitumor activity**
3. **shRNA_TCR, downregulating TCR expression, thus preventing side effects**

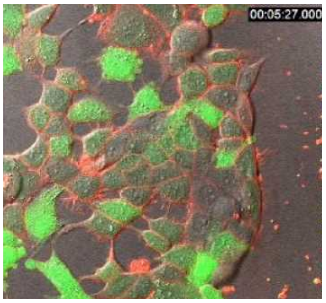
■ **Fig. 22.2. The genetically modified cell lines**



Cell lines, expressing immunosuppresive molecules



The assessment of the immunosuppressive molecules expression levels



CD-19 expression in cells

In total 10 cell lines were genetically modified (such as breast cancer lung cancer, cervical cancer, colorectal cancer)