CLINICAL TRIAL PROTOCOL

Title:	A randomized, multicenter, open-label Phase II trial to compare prophylaxis of graft versus host disease with tacrolimus and mycophenolate mofetil versus ruxolitinib after post-transplant cyclophosphamide
Short title:	PTCy-Ruxo
Version and date:	Draft version 2.0 from 10.10.2019

PROTOCOL SIGNATURE PAGE

Sponsor	Pavlov First Saint Petersburg State Medical University

Ivan Moiseev

Principal investigator	Moiseev I.S, MD, PhD

STUDY PROTOCOL SYNOPSIS

Title	A randomized, multicenter, open-label Phase II trial to compare prophylaxis of graft versus host disease with tacrolimus and mycophenolate mofetil versus ruxolitinib after post-transplant cyclophosphamide
Phase	II
Objectives	• To compare the incidence of acute GVHD grade II-IV; • To compare the non-relapse mortality (NRM); • To compare the incidence of relapse of the underlying disease; • To compare the incidence of chronic, moderate and severe GVHD according to NIH 2015 criteria; • To compare overall survival (OS); • To compare event-free survival (EFS); • To compare the toxicity of two regimens of prophylaxis based • To compare the cumulative incidence of primary graft failure and secondary rejection, not associated with the relapse of the disease; • To compare the incidence of infectious complications.
Endpoints	• Proportion of patients with acute GVHD II-IV grade (timeframe: 125 days); • NRM (timeframe: 2 years); • Proportion of patients with relapse (timeframe: 2 years); • Proportion of patients with chronic, moderate and severe GVHD (timeframe: 2 years); • OS (timeframe: 2 years); • EFS (timeframe: 2 years); • Number of patients with hepatic toxicity (liver function tests), nephrotoxicity (creatinine), cytopenia after engraftment (platelets, white blood cells, neutrophils) are assessed, according to the treating physician neurotoxicity, hemorrhagic cystitis, thrombotic microangiopathy, veno-occlusive disease are assessed (timeframe: 6 months); • Proportion of patients with primary or secondary graft failure (timeframe: 6 months); • Number of patients with bacteremia before engraftment, bacteremia after engraftment, severe sepsis (presence of multiple organ failure), pneumonia, soft tissue infection, invasive mycosis

	(probable or proven invasive aspergillosis, candidaemia, zygomycosis), reactivation of cytomegalovirus, other opportunistic viral infections (timeframe: 6 months).
Study design	This is multicenter investigator-initiated randomized open-label phase II clinical trial to compare prophylaxis of graft versus host disease treated with tacrolimus and mycophenolate mofetil versus ruxolitinib after post-transplant cyclophosphamide. In total 128 patients will be included in the study. After inclusion into the study and performing of transplantation patients will be randomized in 1:1 proportion in two arms (64 patients per arm): arm A will include patients who will be treated with cyclophosphamide and ruxolitinib for GVHD prophylaxis; arm B will include patients who will be treated with cyclophosphamide, tacrolimus and MMF for GVHD prophylaxis. After the end of the treatment patients will be followed-up during two years.
Number of patients	128
Study population	The study will include patients with ALL and AML with indications for transplantation. Indications for transplantation in the first remission for ALL are the presence of a high cytogenetic risk (t(4;11), t(9;22)) and the persistence of minimal residual disease after consolidation as part of program chemotherapy. For acute myeloid leukemia, the indications in the first remission are standard and high cytogenetic risk, patients with translocations t(16;16), t(15;17), t(8;21) are not included. These groups of patients have a 30% increase in relapse-free survival compared with patients without transplantation. All patients in the second remission of ALL and AML (with the exception of t(15;17)) are candidates for HSCT, because programmed chemotherapy, regardless of its options, can achieve no more than 10% of non-progressive survival. In this group of patients, the benefits of performing HSCT are at least 40%. Benefits from HSCT persist even with 15–20% mortality from complications. The highest failure rate of HSCT is associated with a relapse of the disease afterwards. For ALL and AML, methods that

	reduce the risk of relapse are only currently being developed. There are no generally accepted approaches to post-transplant therapy. One of the objectives of this study is to reduce the likelihood of relapse of leukemia.
Inclusion criteria	All eligible patients must meet all the following inclusion criteria: 1. Informed consent to participate in the study, signed by the patient; 2. Diagnosis: acute lymphoblastic or acute myeloblastic leukemia; 3. Morphological remission, defined as less than 5% of blasts by microscopy or flow cytometry with a peripheral leukocyte level of more than 1.500 μL. It is acceptable to include patients without restored platelets or erythrocytes; 4. Indications for performing allogeneic hematopoietic stem cell transplantation, determined by the participating center in accordance with local medical practice; 5. Unrelated or haploidentical donor; 6. Age 18-70 years; 7. Functional status according to ECOG scale 0-2 score.
Exclusion criteria	All eligible patient must not meet any following criteria: 1. Repeated allogeneic transplantation, regardless of the indications for its implementation; 2. Source of graft - umbilical cord stem cells; 3. Any ex vivo modification of the graft with the exception of separation or washing of red blood cells; 4. The presence of more than 5% of clonal tumor cells according to flow cytometry in the presence of morphological remission; 5. Diagnosis: acute promyelocytic leukemia; 6. Severe organ failure: creatinine more than 2 ULN;

	ALT, AST more than 5 ULN; bilirubin more than 1.5 ULN; respiratory failure more than 1 grade; 7. Unstable hemodynamics, requiring the introduction of vasopressors; 8. Uncontrolled bacterial or fungal infection at the time of randomization, determined by the level of CRP> 70 mg/l with adequate antibacterial or antifungal therapy; 9. Arhythmia that persist despite adequate antiarrhythmic therapy: a tachysystolic form of atrial fibrillation, ventricular arrhythmias V gradation according to Laun, AV block of III degree; 10. Decrease in ejection fraction according to echocardiography less than 40%; 11. Angina of more than II functional class or unstable angina; 12. Another severe concomitant pathology, which according to the attending physician does not allow the patient to be included in the study; 13. Pulmonary pathology with a decrease in FEV1 of less than 60% or pulmonary diffusion capacity of less than 60%; 14. Inability to quit smoking for a period of 6 months after transplantation; 15. Pregnancy or refusal to perform highly effective contraception for 6 months after transplantation. 16. Somatic or mental pathology not allowing to sign informed consent.
Investigational medical product	The therapy under investigation is the prophylaxis of GVHD using PTCy and ruxolitinib (PTCy + ruxolitinib). The comparison group is GVHD prophylaxis with the use of PTCy, CNI and MMF (PTCy + CNI + MMF).

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition
AE	adverse event
ALL	acute lymphoblastic leukemia
AML	acute myeloid leukemia
CMV	cytomegalovirus
CNI	calcineurin inhibitors
CRS	cytokine release syndrome
CTCAE	Common Terminology Criteria for Adverse Events
DNA	deoxyribonucleic acid
EFS	event-free survival
GVHD	«graft-versus-host» disease
GVL	«graft-versus-leukemia» effect
HSCT	allogeneic hematopoietic stem cell transplantation
IMP	investigational medical product
MMF	mycophenolate mofetil
NRM	non-relapse mortality
PTCy	post-transplant cyclophosphamide
RNA	ribonucleic acid
SAE	serious adverse event

GLOSSARY OF TERMS

Term	Definition
Acute «graft-versus-host» disease	Develops from the time the transplant is engrafted and up to 100 days after transplantation
Chronic «graft-versus-host» disease	Develops after 100 days from transplantation. In the period from 100-180, there may be an "overlap" syndrome, when GVHD has features of acute and chronic form. In this case, the diagnosis of acute or chronic GVHD is determined by the attending physician, depending on the prevailing clinical manifestations
Disease relapse	In this study, the recurrence of acute leukemia will be considered to be the presence of more than 5% blast cells according to cytology or flow cytometry during bone marrow aspiration after transplantation. Any positive test for minimal residual disease, for which any therapeutic intervention was carried out with the exception of reduction of immunosuppressive therapy, will also be equated with a relapse of the disease
Event-free survival	The time from the moment of transplantation to the moment of death or the end of the observation time, or the recurrence of the disease in accordance with the criteria above
Non-relapse mortality	Mortality from any cause in the absence of data for the relapse of the underlying disease
Overall survival	The time from the moment of transplantation to the time of death or the end of the observation time
Survival without relapse and GVHD	The time from the moment of transplantation to the time of death or the end of the observation period, or recurrence of the disease in accordance with the above criteria, or acute GVHD III-IV grade, or chronic GVHD moderate and severe

1 GENERAL INFORMATION

1.1 Study title, protocol number, version and date

Study title: A randomized, multicenter, open-label Phase II trial to compare prophylaxis of graft versus host disease with tacrolimus and mycophenolate mofetil versus ruxolitinib after post-transplant cyclophosphamide

Phase: II

Protocol number: PTCy-Ruxo

Protocol version: draft 2.0

Protocol date: 10.10.2019

1.2 Responsible parties

Sponsor: Pavlov FSMU

PI: Ivan Moiseev, MD

1.3 Rationale

1.3.1 Investigational medical product

The therapy under investigation is the prophylaxis of GVHD using PTCy and ruxolitinib (PTCy + ruxolitinib). The comparison group is GVHD prophylaxis with the use of PTCy, CNI and MMF (PTCy + CNI + MMF).

1.3.2 Comparator

To date, unrelated and haploidentical transplants prevail in the structure of HSCT (Passweg et al., 2018). However, during transplantation from alternative donors, there is a significant increase in the likelihood of acute and chronic GVHD, which is partially offset by a decrease in the likelihood of relapse. Therefore, overall patient survival is comparable to the results of related matched transplants for most malignant diseases. Anti-thymocyte globulin (ATG) is most often used to reduce the likelihood of lethal GVHD in transplantations from unrelated donors (Bacigalupo et al., 2001). Nevertheless, recent studies have demonstrated the superiority of prophylaxis with PTCy over ATG in unrelated HSCT (Moiseev et al., 2016). At the same time, PTCy is the most commonly used regimen in the world for haploidentical transplants (Luznik et al., 2008). Thus, today the combination of PTCy, CNI and MMF is the most effective prophylaxis in unrelated and haploidentical HSCT. The frequency of clinically significant acute and chronic GVHD usually does not exceed 20–25%, and the mortality rate of GVHD is 5%. Moreover, there are no significant differences in such prophylaxis with regard to GVHD between an unrelated and haploidentical donor (Moiseev et al., 2018). Thus, the best available therapy is used as the comparison group.

1.3.3 Pre-clinical studies

Ruxolitinib was studied in preclinical studies of general pharmacology, safety pharmacology, repeated toxicity, genotoxicity, reproductive toxicity, phototoxicity and carcinogenicity.

Detailed information related with pre-clinical studies described in Investigator's Brochure.

1.3.4 Clinical studies

As of February 22, 2019, more than 9,400 participants in intervention studies have received ruxolitinib. This drug is currently registered in the Russian Federation according to the indications "myelofibrosis" and "polycythemia vera". Please see Investigator's Brochure for detailed information about clinical studies of ruxolitinib.

1.3.5 Risk / benefit

When using the best available prophylaxis that will be used in the comparison group, the mortality from complications of HSCT is about 15%, the risk of relapse in transplantation of acute lymphoblastic leukemia (ALL) and acute myeloblastic leukemia (AML) in 1-2 remissions is 20%. Renal toxicity of grade 2-4 in the comparison group is expected to be at a level of 15%, veno-occlusive liver disease about 3%, thrombotic microangiopathy about 3%. A significant reduction in these complications, a comparable or lower incidence of transplant mortality, and a lower frequency of relapses are expected in the study group. Thus, a 5–10% increase in survival and a better safety profile in the PTCy-ruxolitinib group are expected compared to the best available therapy.

The risks of using PTCy-ruxolinib prophylaxis are associated with a possible increase in the likelihood of severe acute GVHD and mortality associated with it. Based on the available data, it is possible to increase the frequency of acute GVHD II-IV degree by 10%. However, preliminary results suggest a greater likelihood of a response to starting therapy when using this prophylaxis, which eliminates the risk, associated with the greater frequency of this complication. Possible risks include the hematological toxicity of ruxolitinib, delayed engraftment, worse graft function, and a higher incidence of bacterial infections. According to registration studies (Harrison et al., 2012) and the results of using ruxolitinib in the early period after transplantation (Zeiser et al., 2015), hematologic toxicity is expected in 20–30% of patients; however, planned and interventional ruxolitinib dose reductions are prescribed in the protocol, and transplant centers have significant experience in managing patients with poor graft function and pancytopenia, since there are many reasons for poor graft function, including viral infections, antiviral agents toxicity, autoimmune conflicts. On average in the control group, poor graft function is expected in 15% of patients. Thus, even in the case of the development of the hematological toxicity of a number of patients, this is not expected to be accompanied by an increase in the probability of transplant mortality.

1.3.6 Regulatory

The study will be conducted in accordance with this clinical trial protocol, the Helsinki Declaration of the World Medical Association "Ethical Principles for Medical Research with the Human Participation as a Subject" of 1964, with subsequent amendments and additions applicable to the sections of the Federal Law of the Russian Federation 61-FZ "On treatment medicines ", the national standard of the Russian Federation" Good Clinical Practice "(GOST R52379-2005) dated 09/25/2005, order of the Ministry of Health of Russia dated 01.04.2006 No. 200n" On approval of the rules of clinical practice in the Russian Federation ", RF PP No. 714" On approval of standard rules for compulsory life and health insurance for a patient participating in clinical trials of a medicinal product ", Agreement on Uniform Principles and Rules for the Treatment of Medicines in the Framework of the Eurasian Economic Union of 23 December 2014, as well as the ICH GCP guidance documents.

1.3.7 Rationale for dose and regimen selection

Ruxolitinib [(R)-3-(4-(7H-pyrrolo[2,3-d]pyrimidin-4-yl)-1H-pyrazol-1-yl)-3-cyclopentylpropannitrile phosphate] (INCB018424 phosphate, INC424, ruxolitinib phosphate) is a powerful new selective, suitable for oral administration inhibitor of Janus kinase-1 (JAK1) (inhibition concentration by 50% (IC50) = 3.3 ± 1.2 nmol/l) and Janus kinase-2 (JAK2) (IC50 = 2.8 ± 1.2 nmol/l), which has a selective (moderate to profound) effect on Tyrosine kinase-2 (TYK2) (IC50 = 19 ± 3.2 nmol/l) and Janus kinase-3 (JAK3) (IC50 = 428 ± 243 nmol/l), respectively. This drug is currently registered in the Russian Federation according to the indications "myelofibrosis" and "polycythemia vera". In these diseases, a significant component of the clinical manifestations is determined by a mutation in the JAK2 gene, therefore blocking this signaling pathway leads to a decrease in the symptoms of the disease.

However, Janus kinases transmit signals from the main signaling pathways of T-lymphocyte activation, STAT3 and STAT5. It is the activation of these signaling pathways that is one of the main pathogenesis mechanisms of GVHD. In addition, JAK activity regulates antigen presentation by dendritic cells and a number of other immunological processes. Blocking the above mechanisms with ruxolitinib allows you to influence the main pathogenesis mechanisms of GVHD - antigen presentation and proliferation of alloreactive clones, so this drug is used to treat a steroid-refractory form of GVHD (Teshima T, 2016).

To date, one large multicenter retrospective study of the use of ruxolitinib in steroid-refractory acute and chronic GVHD has been published, which showed 82% of the responses in the acute form and 85% of the responses in the chronic form. At the same time, this therapy did not cause a severe decrease in anti-infective immunity, and was accompanied by a relatively low frequency of bacterial and fungal infections. These factors determined extremely favorable overall survival rates for this difficult group of patients (79% and 97% for acute and chronic GVHD, respectively). Although there are currently no prospective comparative studies published with ruxolitinib, however, the results obtained are among the best for such severe complication of

HSCT as steroid-refractory GVHD (Zeiser R, 2015). Similar results were shown in a number of small single-center studies presented at the European Congress of Bone Marrow Transplantation (EBMT Annual Meeting, 2018). In Russian Federation from 2016 to 2018 in Pavlov First Saint Petersburg State Medical University, within the framework of the clinical testing protocol of the Ministry of Health of the Russian Federation (2016-29-1), we also investigated the activity and safety of ruxolitinib in steroid refractory acute and chronic GVHD. According to preliminary results, the response rate in the acute form of GVHD was 83%, and in the chronic form – 85%. The 2-year survival rate for acute GVHD was 58%, while for chronic GVHD – 89%. The data obtained in the protocol of clinical approbation correspond to the data of an international study, which indicates reproducibility of the results and the effectiveness of ruxolitinib in treatment of GVHD.

1.3.8 Study population

The study will include patients with ALL and AML with indications for transplantation. Indications for transplantation in the first remission for ALL are the presence of a high cytogenetic risk (t(4;11), t(9;22)) and the persistence of minimal residual disease after consolidation as part of programmed chemotherapy. For acute myeloid leukemia, the indications in the first remission are standard and high cytogenetic risk, patients with translocations t(16;16), t(15;17), t(8;21) are not included. These groups of patients have a 30% increase in relapse-free survival compared with patients without transplantation. All patients in the second remission of ALL and AML (with the exception of t(15;17)) are candidates for HSCT, because programmed chemotherapy, regardless of its options, can achieve no more than 10% of non-progressive survival. In this group of patients, the benefits of performing HSCT are at least 40%. Benefits from HSCT persist even with 15–20% mortality from complications.

The highest failure rate of HSCT is associated with a relapse of the disease afterwards. For ALL and AML, methods that reduce the risk of relapse are only currently being developed. There are no generally accepted approaches to post-transplant therapy. One of the objectives of this study is to reduce the likelihood of relapse of leukemia.

1.3.9 Study rationale

Allogeneic hematopoietic stem cell transplantation (HSCT) was introduced into medical practice in the 1970s as a treatment of the blood system tumors (Thomas ED, 1975) and various marrow failure syndromes resistant to standard therapy (Thomas ED, 1972). The “graft-versus-host” disease (GVHD) is one of the most frequent and at the same time one of the most life-threatening complications of allogeneic hematopoietic stem cell transplantation (Afanasyev BV, 1997, Savchenko VG, 2007). The main obstacle to the widespread use of this method is transplantation mortality, reaching in some cases 20-30% (Gratwohl A, 2015). The main cause of transplant mortality is GVHD, which develops in 30–70% of patients (Ferrara J, 2004), depending on the type of donor and prophylaxis. In the case of the acute form, mortality can reach 40% (Saliba RM, 2012), and in case of chronic – 10% (Perez-Simon JA, 2008). Despite the emergence of

new methods of treatment, progress in improving the survival of patients with GVHD, especially with the acute form, has been moderate (Gratwohl A, 2015), therefore improving the GVHD prophylaxis protocols is the key to improving the results of transplantation.

Prophylaxis approaches were developed at the Dana-Farber Cancer Center in the 1970s, and included the use of a calcineurin inhibitor, cyclosporin A, in combination with low doses of methotrexate (Storb R, 1989). Despite the introduction of such a scheme more than 40 years ago, a recent study showed that 73% of centers in Europe still use similar prophylaxis (Ruutu T, 2012). Although this type of prophylaxis gives good results in related matched transplantation, allo-HSCT from alternative donors (unrelated and haploidentical) that prevail in the structure of transplantation is associated with a significant incidence of GVHD and high mortality (Beatty PG, 1991). For a long time, the standard for HSCT from alternative donors was the addition of antithymocyte globulin (ATG) to prevention (Bacigalupo, 2001). Nevertheless, in the Russian population of recipients of unrelated HSCT, even with the addition of ATG, the percentage of severe acute and chronic GVHD remained high (Afanasyev BV, 2007, Moiseev IS, 2019). Significant progress in the use of haploidentical or 50% matched donors has been made with the introduction of prophylaxis using post-transplant cyclophosphamide (PTCy) in combination with tacrolimus and mycophenolate mofetil (MMF) (Luznik, 2008). Today, it is the most commonly used in Europe technology of haploidentical transplantation, which allows obtaining clinical results comparable to related compatible transplantation, and is easily reproducible (Lorentino F, 2018). The implementation of this protocol in case of unrelated transplantations in the Russian patient population showed a significant decrease in the incidence of GVHD and transplantation lethality compared with ATG (Moiseev IS, 2016). Thus, among the widely used methods, a combination of PTCy, a calcineurin inhibitor and MMF is currently the most effective prophylaxis of GVHD in unrelated and haploidentical transplantation. The frequency of clinically significant acute and chronic GVHD with its use does not exceed 20% in the Russian patient population (Moiseev IS, 2018). However, this method has several drawbacks: the significant suppression of the “graft-versus-leukemia” effect (GVL), nephrotoxicity observed in 40-50% of patients, the presence of complications associated with calcineurin inhibitors (thrombotic microangiopathy, veno-occlusive liver disease) . In this study, it is planned to show that the combination of PTCy with ruxolitinib shows, at least, not the worse clinical results of HSCT, but reduces the incidence of the above complications.

The profile of complications in patients with GVHD receiving ruxolitinib is extremely favorable. Out of the side effects in patients with acute GVHD, cytopenia was described in 53% of patients, reactivation of cytomegalovirus in 33%. However, cytopenia and viral reactivations are characteristic of this complication and in 51% cytopenia was present before the start of therapy. In chronic GVHD, for which cytopenia is not typical, severe cytopenia was observed in only 7% of patients, which corresponds to the results of the use of the drug in other conditions (Zeiser R, 2016; Harrison C, 2012). Thus, high efficacy against GVHD and a favorable toxicity profile makes ruxolitinib an encouraging candidate to replace calcineurin inhibitors (tacrolimus and

cyclosporine) and MMF in prophylaxis regimens in order to reduce the number of complications and reduce transplant mortality.

Another advantage of using ruxolitinib as opposed to classical immunosuppressants as prophylaxis is the selective suppression of GVHD, without suppressing the GVL, which is the basis of the antitumor effect of allogeneic HSCT (Choi, 2014). Since the majority of patients with HSCT are diagnosed with malignant diseases, and the relapse of the disease is the main cause of mortality after HSCT, maintaining GVHD is the key task of increasing the effectiveness of HSCT. All of the above prerequisites became the basis for Pavlov First Saint Petersburg State Medical University studies on the GVHD prophylaxis with PTCy and ruxolitinib in allogeneic HSCT regarding high-risk myelofibrosis (NCT02806375). A pilot study included 20 patients with myelofibrosis. The results of this study showed satisfactory control of acute GVHD; only 10% of patients required the administration of systemic glucocorticosteroids. No cases of chronic severe GVHD were reported. Only mild nephrotoxicity was observed in 15% of patients and did not require therapy. Cases of veno-occlusive disease are also not recorded. At the same time, not a single case of recurrence of the underlying disease was recorded, although the usual frequency of recurrences of myelofibrosis after HSCT is about 20-30% (Morozova EV, 2017). Thus, the results of the pilot study are extremely encouraging: there is a pronounced antitumor activity of this prevention option and a favorable toxicity profile, which creates prerequisites for conducting a multicenter validation study.

The choice of dose is based on the results of the pilot Pavlov First Saint Petersburg State Medical University study (NCT02806375) and clinical trials of the Ministry of Health of the Russian Federation (2016-29-1). In the NCT02806375 study, the starting dose of ruxolitinib was 15 mg, while 40% of patients required dose reduction after 30 days due to poor graft function. The pharmacokinetics in this study showed accumulation of ruxolitinib with daily intake and a 3-fold increase in the minimum concentration (C0) after 2 and 10 days of administration, respectively. Also in clinical trials using doses of 20 mg for the treatment of moderate GVHD, grade 3-4 hematologic toxicity was observed in 30% of patients, which indicates the need to use doses less than 20 mg to ensure safe engraftment. Also, to prevent poor graft function and associated mortality, from the 21st day a dose reduction of up to 10 mg per day is planned. The results of the NCT02806375 study showed that reducing the dosage to 10 mg due to poor graft function did not lead to a decrease in the control of GVHD. In these patients, there were no episodes of acute GVHD after dose reduction. Also in this study, therapy with ruxolitinib was performed during the conditioning to control the manifestations of the underlying disease. However, it is known that ruxolitinib administration prior to HSCT reduces the likelihood of GVHD afterwards. It is possible that the favorable control of GVHD was also associated with the pre-transplant administration of ruxolitinib (Kroeger et al., 2018), therefore, in this study, the use of ruxolitinib during the conditioning at a dose of 15 mg was left unchanged. Thus, on the basis of the available data, the optimal dosage regimen is the starting dosage of 5 mg 3 times a day

during the conditioning, then – from the day +5, followed by reduction to 5 mg 2 times a day by day +21 HSCT.

The effect of PTCy is based on the activation of T cells in the absence of immunosuppression immediately after transplantation and selective apoptosis of activated alloreactive cells (Luznik L, 2012). It is known that using PTCy as the only component of GVHD prophylaxis may be sufficient to control GVHD with related matched bone marrow transplantation (Luznik L, 2010), however, with the use of peripheral blood stem cells (PBSC), transplantation from unrelated and haploidentical donors who make up a large part in the structure of transplantation activity, PTCy alone is not enough to prevent severe GVHD manifestations (Holtick U, 2016), therefore the combination of other immunosuppressive drugs should be used in such situations. Most centers use calcineurin inhibitors (CNIs) (cyclosporin A or tacrolimus) and MMF. This combination is planned as a control group in this study. This type of prophylaxis for unrelated and haploidentical transplants provides good control of acute GVHD (less than 20% has clinically significant manifestations) and only 15% of patients have symptoms of moderate to severe chronic GVHD. There is also a low level of non-relapse mortality, not exceeding 10% in the group of patients with standard risk. At the same time, there are no significant differences between unrelated and haploidentical donors. Nephrotoxicity in 40% of patients is a negative aspect of this prophylaxis regimen, and hemorrhagic cystitis rate is 15%. Also, it does not increase the incidence of GVHD compared with the classic prophylaxis regimens, and the relapse of the disease is the main cause of treatment failure (Moiseev IS, 2018).

Considering the extremely high efficacy of GVHD control when using the PTCy-CNI-MMF regimen, with any realistic sample size, it will be impossible to demonstrate the advantages of the PTCy-ruxolitinib regimen in terms of control of acute and chronic GVHD. In addition, the results of a pilot study showed a similar incidence of acute and chronic GVHD with the PTCF-CNI-MMF regimen. Therefore, the primary goal is to demonstrate the equivalence of PTCF-CNI-MMF and PTCy-ruxolitinib in terms of GVHD control. Since GVHD, unlike nephrotoxicity and most cases of endothelial complications of CNI, is a potentially life-threatening event, it is first necessary to demonstrate equivalent GVHD control when using these two types of prophylaxis.

The secondary tasks, first of all, include demonstration that the non-relapse mortality (NRM) using the PTCy-ruxolitinib mode will not be significantly worse. Considering that in a pilot study (Morozova EV, 2017) NRM in such a severe HSCT patient group as myelofibrosis was 15%, it is expected that mortality will also be comparable when using these two protocols. The second important secondary point is the relapse rate. At present, there are no data in the literature on the frequency of relapses in the PTCy-ruxolitinib group, however, the probability of relapse of the underlying disease is expected to decrease at least two-fold. Other secondary points for which significant differences are expected are the frequency of nephrotoxicity and endothelial complications (veno-occlusive liver disease and thrombotic microangiopathy). The remaining rates of overall survival and event-free survival stem from the above objectives. Survival rates

are expected to be at least as good in the PTCy-ruxolitinib group as in the PTCy-CNI-MMF group. In case of a decrease in the frequency of relapses in the PTCy-ruxolitinib group, an improvement in survival without relapse is expected.

2 STUDY OBJECTIVES

The main study aim is to compare the efficacy and safety of GVHD prophylaxis with PTCy and ruxolitinib to control group where prophylaxis will be carried out with PTCy, CNI and MMF.

Primary study objective is:

- To compare the incidence of acute GVHD.

Secondary study objectives are:

- To compare the non-relapse mortality (NRM)
- To compare the incidence of relapse of the underlying disease
- To compare the incidence of chronic, moderate and severe GVHD according to NIH 2015 criteria
- To compare overall survival (OS)
- To compare event-free survival (EFS)
- To compare the toxicity of two regimens of prophylaxis based
- To compare the cumulative incidence of primary graft failure and secondary rejection, not associated with the relapse of the disease
- To compare the incidence of infectious complications

3 STUDY DESIGN

3.1 Investigational parameters (endpoints)

Primary endpoint of this study is:

- Proportion of patients with acute GVHD II-IV grade (timeframe: 125 days).

Secondary endpoints of this study are:

- NRM (timeframe: 2 years)
- Proportion of patients with relapse (timeframe: 2 years)
- Proportion of patients with chronic, moderate and severe GVHD (timeframe: 2 years)
- OS (timeframe: 2 years)
- EFS (timeframe: 2 years)

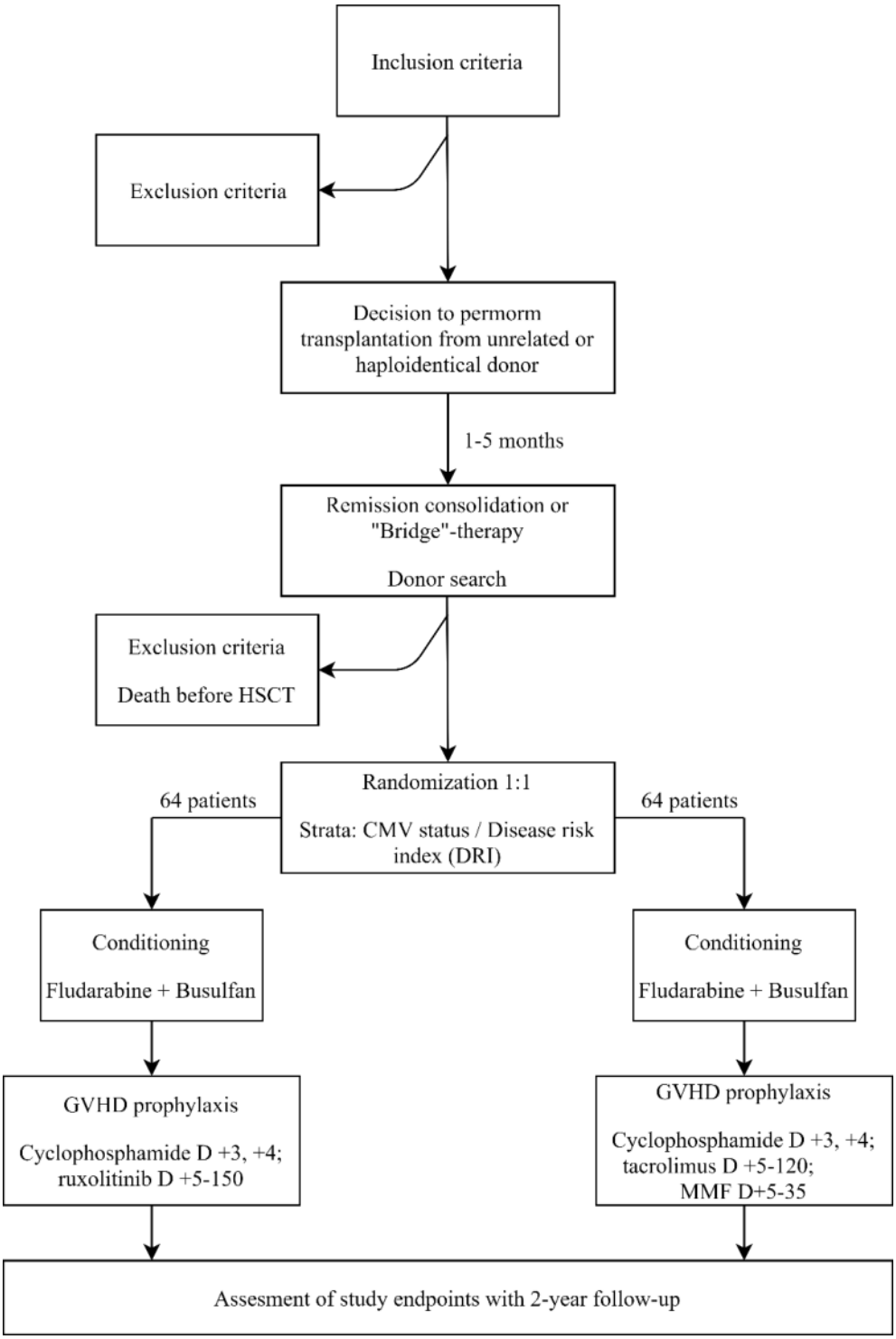
- Number of patients with hepatic toxicity (liver function tests), nephrotoxicity (creatinine), cytopenia after engraftment (platelets, white blood cells, neutrophils) are assessed, according to the treating physician neurotoxicity, hemorrhagic cystitis, thrombotic microangiopathy, veno-occlusive disease are assessed (timeframe: 6 months)
- Proportion of patients with primary or secondary graft failure (timeframe: 6 months)
- Number of patients with bacteremia before engraftment, bacteremia after engraftment, severe sepsis (presence of multiple organ failure), pneumonia, soft tissue infection, invasive mycosis (probable or proven invasive aspergillosis, candidaemia, zygomycosis), reactivation of cytomegalovirus, other opportunistic viral infections (timeframe: 6 months)

3.2 Overall study design

This is multicenter investigator-initiated randomized open-label phase II clinical trial to compare prophylaxis of graft versus host disease treated with tacrolimus and mycophenolate mofetil versus ruxolitinib after post-transplant cyclophosphamide.

Figure 3-1 summarizes the study design.

Figure 3-1: Study design


Inclusion criteria
Exclusion criteria
Decision to permorm transplantation from unrelated or haploidentical donor
1-5 months
Remission consolidation or "Bridge"-therapy
Donor search
Exclusion criteria
Death before HSCT
Randomization 1:1
Strata: CMV status / Disease risk index (DRI)
64 patients
64 patients
Conditioning
Fludarabine + Busulfan
Conditioning
Fludarabine + Busulfan
GVHD prophylaxis
Cyclophosphamide D +3, +4; ruxolitinib D +5-150
GVHD prophylaxis
Cyclophosphamide D +3, +4; tacrolimus D +5-120; MMF D+5-35
Assesment of study endpoints with 2-year follow-up

3.3 Randomization

After inclusion into the study and performing of transplantation patients will be randomized in 1:1 proportion in two arms (64 patients per arm): arm A will include patients who will be treated with cyclophosphamide and ruxolitinib due to GVHD prophylaxis; arm B will include patients who will be treated with cyclophosphamide, tacrolimus and MMF due to GVHD prophylaxis. After the end of the treatment patients will be followed-up during two years.

3.4 Masking

Not applicable due to open-label desigh.

3.5 Number of subjects

128 patients will be included in the study.

3.6 Methodology

During the course of the study the allogeneic hemopoietic stem cell transplant procedure is performed for all participating patients. The transplant procedure includes the following stages:

- Conditioning regimen consisting of fludarabin and busulfan performed from D-7 to D-1
- The graft transfusion at D0
- Post-transplant cyclophosphamide infusion as part of GVHD prophylaxis
- Imunosuppressive therapy from D+5 to D+120 in control group and D+150 in study group

The pre-engraftment stage is delivered in inpatient conditions in accordance with Russian Health Ministry regulation on healthcare delivery in patients with conditions, in which the bone marrow and hemopoietic stem cells transplantation are indicated as well as the updates to healthcare delivery Regulation in accordance with “surgery (human organs and/or tissues transplantation)”.

The post-engraftment treatment may be delivered in inpatient, day hospital or outpatient conditions. The number of visits after the patient is discharged from the hospital is regulated by internal standard procedures of participating sites. The visits on day 100, day 180, 1 year, and 2 years after the transplant (final visit) are mandatory.

Table 3-1: Study visits

Study visits / Study procedures	**SCR**	**ASCT visit**	**Prophylaxis visits**	**Follow-up visits**		
Visit:		**V^1**	**V^2**	**V^3**	**V^4**	**V^5**
Day:		**D^0**	**D^{100}**	**D^{180}**	**$Y1D^5_{15}$**	**$Y2D^{880}$**
Eligibility criteria	×					

Study visits / Study procedures	**SCR**	**ASCT visit**	**Prophylaxis visits**	**Follow-up visits**		
Visit:		**V[1]**	**V[2]**	**V[3]**	**V[4]**	**V[5]**
Day:		**D[0]**	**D[100]**	**D[180]**	**Y1D[5] 15**	**Y2D[880]**
ICF signing	×					
Demographics / anthropometrics data	×					
HIV, HCV, HBV test	×					
Pregnancy test	×					
Complete blood count	×		×[c]	×		
Blood chemistry	×		×	×		
Electrocardiography	×					
Echocardiography	×					
Microscopy or flow cytometry	×					
CMV test[a]	×					
Graft composition[b]		×				
Spirography	×					
Physical examination / ECOG	×		×	×	×	×
Comorbidities	×					
Disease risk index	×					
HCT-CI	×					
C-reactive protein test						
ASCT		×				
Acute GVHD assessment			×			
Chronic GVHD assessment				×	×	×
Serum EDTA	×	×	×	×		
Blood DNA and RNA	×		×			
Chimerism test[d]			×	×[e]	×[e]	×[e]
Bone marrow biopsy			×	×		
Relapse assessment			×	×	×	×
Adverse event collection			×	×	×	×
Secondary malignancies					×	×

a for assessment of recipient / donor CMV serostatus;
b Minimally CD34+, NC, CD3+;
c for assessment of WBC, neutprophils and PLT engraftment;
d by CRP or the relevant method;
e not mandatory

3.6.1 Screening

The following procedures will be performed on the screening visit:

- Patient written informed consent for study participation must be obtained before any study-related procedures;
- Check for eligibility criteria;
- Collection of demographic and anthropometric data;
- HIV, HCV, HBV test (may be performed during one month before screening);
- Pregnancy test for woman patients;
- Complete blood count;
- Blood chemistry;
- Echocardiography at rest;
- Physical examination and patient condition according to ECOG scale.
- Comorbidities;
- DRI and HCT-CI calculation.

3.6.2 Biological samples cryopreservation

The current study involves blood ruxolitinib concentration and cytokines levels determination. Also there are plans for blood Janus kinases levels assessment. Table 3-2 summarizes the schedule for obtaining samples to be cryocopreserved. The collection is not mandatory and will be performed by the centers willing to participate in biologic research studies after the completion of the protocol.

Table 3-2: Cryopreservation schedule

Sample / Visit	**V1**	**V8**	**V10**	**V11**	**V12**	**V13**	**V14**	**V15**	**V17**
Day:	**D-7**	**D0**	**D7**	**D14**	**D21**	**D30**	**D60**	**D100**	**D180**
Serum EDTA	×	×	×	×	×	×	×	×	×
DNA and RNA peripheral blood	×					×	×	×	×

Plasma processing: the sample (it should not be taken from the first portion of blood) is obtained into EDTA-containing test tube from central venous catheter or peripheral vein. During 2-3 hours after the sample was obtained it is centrifuged at 4°C and 1000g for 15 minutes. Plasma is

aliquoted as 1 ml portions into at least 2 separate samples. Aliquoted samples are frozen at -70-80°C until the day the test is performed.

DNA processing: the sample (it should not be taken from the first portion of blood) is obtained into EDTA-containing test tube from central venous catheter or peripheral vein. During the same day the DNA and RNA are purified using a standard method, then samples are frozen at -20-40°C Ountil the day the test is performed. If DNA and RNA purification is impossible, then whole blood samples may be frozen in cryogenic test tube at -20-40°C for further study.

3.6.3 Transplantation visit

The following procedures will be performed during this visit:

- Graft composition evaluation
- ASCT.

3.6.4 Prophylaxis and Follow-up visits

The following procedures will be performed during follow-up visits:

- Physical examination and patient condition according to ECOG scale.
- Chimerism assessment
- Relapse assessment by bone marrow or peripheral blood morphology, MRD testing will be performed only as part of the center routine clinical practice.
- Acute GVHD staging on V2 (maximal clinical grades recorded)
- Chronic GVHD assessment and staging if necessary
- Adverse event collection
- Secondary malignancies

3.6.5 Early termination

The patients may stop receiving study therapy at any time for any reason. If a patient decides to stop receiving study therapy, than the researcher should deploy reasonable efforts (i.e. making a phone call or sending a message/e-mail) to determine the main reason for this decision. If the patient informs of an intention to withdraw his consent, stops following visits schedule, or become lost to follow-up due to any other reason, than this should be considered leaving the study.

The investigator may cease study therapy if he decides its continuation is harmful for a patient and may lead to deterioration of his condition. The study therapy cessation should also be considered in the following situations:

- Development of a life-threatening complication (Gr.4 according to CTCAE 5.0) except hematological toxicity, which is related to study therapy according to researcher's opinion

- Underlining condition relapse or progression
- Signs of graft rejection requiring rapid immunosuppressive therapy withdrawal and an unplanned donor lymphocyte infusion, “boost” with donor stem cells or other anticancer therapy
- Pregnancy
- Protocol deviations possible compromising the patient’s safety including the intake of forbidden medications

Patients, which cease study therapy are NOT considered leaving the study, they should continue visits for evaluation (see Section 5.1) to be transferred to long-term survival evaluation group. If the patients cease visits due to unknown reason, than the researcher should deploy reasonable efforts (i.e. making a phone call or sending a letter/e-mail) to contact patient.

3.6.6 Subject withdrawal

All patients may withdraw their consent for study participation at any time and for any reason. The only case, in which the consent is withdrawn, is when a patient clearly refuses to take part in the study further, declines any further evaluation or visits, as well as all contacts related to the study.

The study sponsor will keep all records and study results, which were already obtained for evaluation. All biological samples collected may be kept for subsequent evaluation (or any other use corresponding to legal requirements).

In case of consent withdrawal the researcher should deploy reasonable efforts (i.e. making a phone call or sending a letter/e-mail) to determine and document the main reason for this decision. In this case the study therapy should be stopped and an alternative GVHD prophylaxis regimen in accordance with standard operation procedures of this participating center should be offered.

3.7 Study termination

Intermediate results of the study are presented annually to the heads of transplant centers participating in the study. Based on the submissions, a decision is made on the safety of continuing the study. Recommended event thresholds in the study group for stopping the study after the first year of inclusion (20 patients in the study group: more than 50% acute GVHD II – IV degree requiring systemic glucocorticosteroid therapy, more than 50% chronic GVHD moderate and severe, more than 30% NRM). Recommended event thresholds in the study group for discontinuing the study after the second year of inclusion (40 patients in the study group): more than 40% acute GVHD grade II-IV requiring systemic glucocorticosteroid therapy, more than 40% of chronic GVHD is moderate to severe, more than 25% NRM. The decision to terminate the study should be adopted by the supervisory board collectively. In the case of the

decision to change the protocol instead of the termination of the study, the changes must be submitted for approval by the ethics committees of participating centers.

The criterion for changing the dosing regimen of ruxolitinib is the presence of more than 30% of severe poor graft function during interim analysis. The decision to change the dosage is also made by supervisory board and is submitted by the ethical committees.

The composition of the supervisory board of the study:

- Pavlov First Saint Petersburg State Medical University: Afanasyev, Boris Vladimirovich, Director of the Scientific and Research Institute for Children's Oncology, Hematology and Transplantology named after R.M. Gorbacheva, MD, Honored Doctor of Russia;
- National Research Center for Hematology: Elena Nikolayevna Parovichnikova, Head of the Department of Chemotherapy of Hemoblastosis, Hematopoietic Depression and BMT, Doctor of Medicine;
- The Federal State-Financed Scientific Institution Kirov Research Institute of Hematology and Blood Transfusion under the Federal Medical Biological Agence (KRIHBT): Paramonov Igor Vladimirovich, Director of the Research Institute of Hematology and Blood Transfusion, MD.

3.8 Drug storage and accountability

The IMP must be kept in a secured location in storage conditions according to its SmPC. Investigator must keep all related drug supplies documentation.

Quantity of IMP packages and its administration to patients must be fulfilled in IMP accountability log by responsible site person.

Each patient must keep a IMP accountability diary. Patient’s IMP compliance will be assessed through each study visit by the investigator.

4 STUDY POPULATION

4.1 Inclusion criteria

All eligible patients must meet all the following inclusion criteria:

1. Informed consent to participate in the study, signed by the patient;
2. Diagnosis: acute lymphoblastic or acute myeloblastic leukemia;
3. Morphological remission, defined as less than 5% of blasts by microscopy or flow cytometry with a peripheral leukocyte level of more than 1.500 μL. It is acceptable to include patients without restored platelets or erythrocytes;
4. Indications for performing allogeneic hematopoietic stem cell transplantation, determined by the participating center in accordance with local medical practice;

5. Unrelated or haploidentical donor;
6. Age 18-70 years;
7. Functional status according to ECOG scale 0-2 score.

4.2 Exclusion criteria

All eligible patient must not meet any following criteria:

1. Repeated allogeneic transplantation, regardless of the indications for its implementation;
2. Source of graft - umbilical cord stem cells;
3. Any ex vivo modification of the graft with the exception of separation or washing of red blood cells;
4. The presence of more than 5% of clonal tumor cells according to flow cytometry in the presence of morphological remission;
5. Diagnosis: acute promyelocytic leukemia;
6. Severe organ failure: creatinine more than 2 ULN; ALT, AST more than 5 ULN; bilirubin more than 1.5 ULN; respiratory failure more than 1 grade;
7. Unstable hemodynamics, requiring the introduction of vasopressors;
8. Uncontrolled bacterial or fungal infection at the time of randomization, determined by the level of CRP> 70 mg/l with adequate antibacterial or antifungal therapy;
9. Rhythm disturbances that persist despite adequate antiarrhythmic therapy: a tachysystolic form of atrial fibrillation, ventricular arrhythmias V gradation according to Laun, AV block of III degree;
10. Decrease in ejection fraction according to echocardiography less than 40%;
11. Angina of more than II functional class or unstable angina;
12. Another severe concomitant pathology, which according to the attending physician does not allow the patient to be included in the study;
13. Pulmonary pathology with a decrease in FEV1 of less than 60% or pulmonary diffusion capacity of less than 60%;
14. Inability to quit smoking for up to 6 months after transplantation;
15. Pregnancy or refusal to perform highly effective contraception for 6 months after transplantation.

    Highly effective contraceptive methods include:

- Total abstinence: if it corresponds to the preferred and customary way of life of the patient. Periodic abstinence (for example, calendar, ovulation, symptothermal, postovulation methods) and interrupted sexual intercourse are not considered acceptable methods of contraception;

- Female sterilization (surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy or tubal ligation at least 6 weeks before the start of the therapy being studied. In the case of ovariectomy only, the reproductive status of the woman must be confirmed using a subsequent analysis of hormones;

- Sterilization of the male partner (at least 6 months before screening). For women participating in the study, the sexual partner after a vasectomy should be the only partner;

- Use of oral, injectable or implanted hormonal contraceptive drugs, intrauterine devices or contraceptive systems, or other forms of hormonal contraception with similar efficacy (failure rate less than 1%), for example, hormonal vaginal rings or transdermal hormonal contraceptives.

16. Somatic or mental pathology not allowing to sign informed consent.

5 STUDY TREATMENTS

5.1 Allocation to treatment

Patients will be randomized before the start of conditioning based on 2 strata – type of donor and a disease risk index (DRI). Detailed mathematical methods for randomization are described in Section 2.5, Statistical Methods. Stratification will be site-specific, that means each site will have an equal number of patients in the study arms.

5.2 Conditioning therapy

Mandatory for inclusion in this study is the conditioning therapy, which includes administration of two medications – fludarabine and busulfan. The total dose of fludarabine 180 mg/m^2 during 6 days, i.e. daily dose of 30 mg/m^2. The dose and route of administration of busulfan are selected within the framework of the standard operating procedures of the investigational sites depending on the age, patient's somatic status, comorbidity, and tolerability of prior chemotherapy.

Possible regimes conditioning therapy are summarized in Table 5-1, Table 5-2, Table 5-3, Table 5-4 and Table 5-5.

Table 5-1: Busulfan 8 mg/kg conditioning regime

Drug	Dose	Route of administration		Days							
				D -7	D -6	D -5	D -4	D -3	D -2	D -1	SCT
Fludarabine	30 mg/m^2/day	iv	days -7 to -2, 1-hour infusion	×	×	×	×	×	×		
Busulfan[1]	4 mg/kg/day	p/o	days -4 to -3 p/o at 06^{00}, 12^{00}, 18^{00} and 24^{00}				××××	××××			
Ruxolitinib	15 mg/day	iv	days -7 to -2 from +5 p/o in three doses	×	×	×	×	×	×		

1 Busulfan 8 mg/kg: 1 mg/kg per os 4 times a day, days -4 and -3 before transplantation

Table 5-2: Busulfan 10 mg/kg conditioning regime

Drug	Dose	Route of administration		Days							
				D -7	D -6	D -5	D -4	D -3	D -2	D -1	SCT
Fludarabine	30 mg/m^2/day	iv	days -7 to -2, 1-hour infusion	×	×	×	×	×	×		
Busulfan[1]	4 mg/kg/day	p/o	day -5 p/o at 18^{00} and 24^{00}; days -4 to -3 p/o at 06^{00}, 12^{00}, 18^{00} and 24^{00}			××	××××	××××			
Ruxolitinib	15 mg/day	iv	days -7 to -2 from +5 p/o in three doses	×	×	×	×	×	×		

1 Busulfan 10 mg/kg: 1 mg/kg per os 4 times a day, days -5, -4 and -3 before transplantation. In D-5, the patient performs only 2 evening medications

Table 5-3: Busulfan 12 mg/kg conditioning regime

Drug	Dose	Route of administration		Days							
				D -7	D -6	D -5	D -4	D -3	D -2	D -1	SCT
Fludarabine	30 mg/m^2/day	iv	days -7 to -2, 1-hour infusion	×	×	×	×	×	×		
Busulfan[1]	4 mg/kg/day	p/o	days -5 to -3 p/o at 06^{00}, 12^{00}, 18^{00} and 24^{00}			××××	××××	××××			
Ruxolitinib	15 mg/day	iv	days -7 to -2 from +5 p/o in three doses	×	×	×	×	×	×		

1 Busulfan 12 mg / kg: 1 mg / kg per os 4 times a day at Day -5, Day -4 and Day -3

Table 5-4: Busulfan 14 mg/kg conditioning regime

Drug	Dose	Route of administration		Days							
				D -7	D -6	D -5	D -4	D -3	D -2	D -1	SCT
Fludarabine	30 mg/m^2/day	iv	days -7 to -2, 1-hour infusion	×	×	×	×	×	×		
Busulfan[1]	4 mg/kg/day	p/o	day -6 at 18^{00} and 24^{00}; days -5 to -3 p/o at 06^{00}, 12^{00}, 18^{00} and 24^{00}		××	××××	××××	××××			
Ruxolitinib	15 mg/day	iv	days -7 to -2 from +5 p/o in three doses	×	×	×	×	×	×		

1 Busulfan 14 mg / kg: 1 mg / kg per os 4 times a day, D-6, -5, -4, -3. In D-6, the patient performs only 2 evening medications

Table 5-5: Busulfan 16 mg/kg conditioning regime

Drug	Dose	Route of administration		Days							
				D -7	D -6	D -5	D -4	D -3	D -2	D -1	SCT
Fludarabine	30 mg/m^2/day	iv	days -7 to -2, 1-hour infusion	×	×	×	×	×	×		
Busulfan[1]	4 mg/kg/day	p/o	days -6 to -3 p/o at 06^{00}, 12^{00}, 18^{00} and 24^{00}		××××	××××	××××	××××			
Ruxolitinib	15 mg/day	iv	days -7 to -2 from +5 p/o in three doses	×	×	×	×	×	×		

1 Busulfan 16 mg / kg: 1 mg / kg per os 4 times a day, D-6, -5, -4, -3

Calculation of doses of busulfan is carried out on the adjusted weight with a difference between real and ideal weight of more than 20%. The formula of ideal weight for men is 50 + 0.91 * ("height" -152), for women 45 + 0.91 * ("height" -152). The formula for the adjusted weight for men is "ideal weight" +0.25 * ("real weight" - "ideal weight"), for women "ideal weight" +0.25 * ("real weight" - "ideal weight"). Investigational sites will be provided with a spreadsheet for automatic calculation of doses of medications for conditioning regimen and GVHD prophylaxis.

If there is a concomitant pathology that does not allow for conditioning using busulfan, it is possible to use alternative regimens with prior agreement with the principal investigator.

Oral busulfan can be replaced with an intravenous form (currently not registered in the Russian Federation). The dose conversion factor for intravenous form is 0.8. Thus, the total intravenous dose for the recommended regimen with reduced toxicity is 6.4 mg / kg, and for the myeloablative one it is 11.2-12.8 mg / kg. The frequency of administration for this protocol is 4 times a day.

5.3 Prophylaxis of graft-versus-host disease

5.3.1 PTCy + CNI + MMF

There are two possible treatment regimen for prophylaxis of GVHD in PTCy + CNI + MMF arm.

First one:

- Cyclophosphamide 50 mg kg at D+3, D+4 and 500 ml 0.9% NaCl intravenous. in 2 hours. Calculation of doses of cyclophosphamide is carried out on the adjusted weight with a difference between real and ideal weight of more than 20%.
- Uromitexan 100% of the dose of cyclophosphamide, 24-hour infusion, starting 3 hours before the administration of cyclophosphamide, ending 24 hours after the end of the administration. It is acceptable to increase the dose of uromitexan up to 200% of the dose of cyclophosphamide with the development of abdominal pain syndrome or cystalgia during the administration. Calculation of doses of uromitexan is always carried out on real weight.
- Tacrolimus 0.03 mg/kg intravenous 24-hour infusion from D+5. In the presence of antifungal prophylaxis with fluconazole, the dose is reduced by 25%, with the use of voriconazole by 50%. Transfer to the oral form is carried out after the transplant engraftment. Tacrolimus concentration target values are 3-15 ng/ml.

Second one:

- Cyclosoprin A 1.5 mg/kg and 100-250 ml 0.9% NaCl intravenous in 2 hours 2 times a day from D +5. Transfer to the oral form is carried out after the transplant engraftment. Cyclosoprin A concentration target values of 150-350 ng/ml.
- Mycophenolate mofetil 30 mg/ kg/day per os with 10/10 HLA compatible donor and 45 mg/kg/day per os with <10/10 HLA compatible donor from D+5 to D+35. With the development of mucositis, dispersion of tablets or the use of an intravenous form in a 1: 1 ratio is permissible.

Calcineurin inhibitors are continued until D+100, when the dose is gradually tappered in accordance with the standard medical practice of the participating center. In the absence of GVHD D+150, calcineurin inhibitors should be discontinued. Recommendations for dose adjustment of calcineurin inhibitors and transition from intravenous to oral form are presented in Appendix 1.

Introduction of glucocorticosteroids: in the absence of life-threatening conditions, it is highly desirable to avoid the introduction or administration of glucocorticosteroids from D-5 to D+5. The administration of glucocorticosteroids outside this time interval is not limited. After a transfusion of the graft, the patient may develop cytokine release syndrome (CRS). Most often,

the CRS is resolved on D+3 after the introduction of cyclophosphamide without specific therapy. Staging of the CRS and procedure are presented in Appendix 2. Transfusion of the graft is performed WITHOUT premedication with glucorticosteroids, and with premedication with dimedrol and metamizole, or without premedication. Management of mild transfusion complications and mild episodes of hemolysis is also performed without glucorticosteroids.

5.3.2 RTCy + ruxolitinib

- Cyclophosphamide 50 mg / kg D+3, D+4 + 500 ml 0.9% NaCl intravenous in 2 hours. Calculation of doses of cyclophosphamide is carried out on the adjusted weight with a difference between real and ideal weight of more than 20%.
- Uromitexan 100% of the dose of cyclophosphamide, 24-hour infusion, starting 3 hours before the administration of cyclophosphamide, ending 24 hours after the end of the administration. It is acceptable to increase the dose of uromitexan up to 200% of the dose of cyclophosphamide with the development of abdominal pain syndrome or cystalgia during the administration. Calculation of doses of uromitexan is always carried out on real weight.
- Ruxolitinib 5 mg 3 times a day from D-7 to D-2.
- Ruxolitinib 5 mg 3 times a day from D+5 to D+21.
- Ruxolitinib 5 mg 2 times a day from D+22 to D+150.

Introduction of glucocorticosteroids: in the absence of life-threatening conditions, it is highly desirable to avoid the introduction or administration of glucocorticosteroids from D-5 to D+5. The administration of glucocorticosteroids outside this time interval is not limited. After a transfusion of the graft, the patient may develop cytokine release syndrome (CRS). Most often, the CRS is resolved on D+3 after the introduction of cyclophosphamide without specific therapy. Staging of the CRS and procedure are presented in Appendix 2. Transfusion of the graft is performed WITHOUT premedication with glucorticosteroids, and with premedication with dimedrol and metamizole, or without premedication. Management of mild transfusion complications and mild episodes of hemolysis is also performed without glucorticosteroids.

5.4 Acute graft-versus-host disease treatment

The morphological verification is not required prior to skin and liver acute GVHD therapy initiation. However, it is necessary before gut GVHD treatment starts if there are no other clinical signs. If the patient has both skin and/or liver involvement signs requiring systemic therapy and gut involvement the latter may be verified morphologically at the moment of treatment initiation or later during its course.

Grade II-IV acute GVHD treatment in control group (PTCy+CNI+MMF) is preformed according to local guidelines and standards of investigational sites.

For grade II-IV acute GVHD treatment in PTCy+ruxolitinib group there is a good chance of obtaining complete response without administration of systemic steroids:

- For grade III acute skin GVHD *without* accompanying grade 3-4 cytopenia the daily ruxolitinib dose is increased to 20 mg;
- If the patient has grade III acute skin GVHD *and* accompanying grade -4 cytopenia he/she should be given cyclosporine-A 3 mg/kg or tacrolimus 0.03 mg/kg intravenous or per os;
- Patients with grade III acute skin GVHD *without* response to increased ruxolitinib dose are given cyclosporine-A 3 mg/kg or tacrolimus 0.03 mg/kg intravenous or per os;
- Patients with grade III acute skin GVHD *without* response to increased ruxolitinib dose and CNIs are treated according to investigational sites' guidelines;
- Grade I acute gut or hepatic GVHD *without* any accompanying grade 3-4 cytopenia requires an increase of daily ruxolitinib dose to 20 mg;
- If the patient has grade I acute gut or hepatic GVHD *with* any accompanying grade 3-4 cytopenia he/she should be given cyclosporine-A 3 mg/kg or tacrolimus 0.03 mg/kg intravenous or per os;
- Patients with grade I acute gut or hepatic GVHD *without* response to increased ruxolitinib dose are given cyclosporine-A 3 mg/kg or tacrolimus 0.03 mg/kg intravenous or per os;
- Patients with grade I acute gut or hepatic GVHD *without* response to increased ruxolitinib dose and CNIs are treated according to investigational sites' guidelines;
- Grade IV acute skin GVHD and/or grade ≥ II gut GVHD and/or grade ≥ II hepatic GVHD is an indication for oral or iv cyclosporine-A 3 mg/kg or tacrolimus 0.03 mg/kg + systemic steroids in accordance to investigational sites' guidelines;

Ruxolitinib is given up to D+150 after complete GVHD response registration and until all other immunosuppressive medication are withdrawn.

5.5 Dose regimen and modification

Rationale for dose adjustment of ruxolitinib:

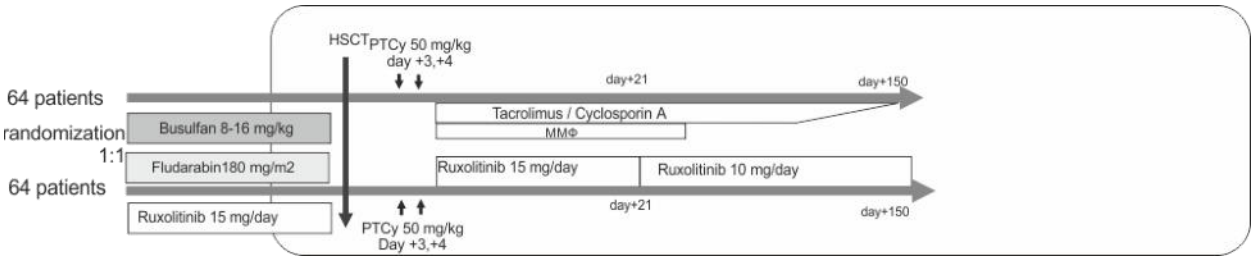
1. The presence of thrombocytopenia, leukopenia or neutropenia of 4 grade after the 50th day from transplantation, with no other identified causes, except for toxicity: reduction to 2.5 mg BID.
2. Life-threatening viral infection in the presence of GVHD manifestations: reduction to 2.5 mg BID to D + 60 or cancellation after D + 60.

3. Detectable minimal residual disease: reduction to 2.5 mg BID to D + 60 or cancellation after D + 60.

4. Acute kidney injury after conditioning or graft transfusion or treatment with reduced GFR less than 30 ml/m^2/min: 5 mg 2 BID, further correction for hematological toxicity after engraftment. In the case of a decrease in GFR of less than 15 ml/m^2/min, it is recommended to conduct dialysis on D +5, taking into account the likelihood of occurrence of complications not only due to ruxolitinib, but also due to PTCy.

Figure 5-1 summarizes the possible dose modifications.

Figure 5-1: Dose regimen and modifications



Postponement of the onset of prophylaxis with ruxolitinib is not allowed with the development of any complications of therapy.

5.6 Drug supplies

The investigational medical product (IMP) ruxolitinib will be labeled and provided by Novartis Company.

IMP's label will contain all necessary legal information such as drug name, batch/series number, expiration date, qualitative/quantitative composition, administration guidelines, storage conditions and patient identification code, which one must be fulfilled by site responsible person.

5.7 Other study treatments

All other study treatments defined in Sections 6.1, 6.2, 6.3, 6.4 and 6.5 will be provided to patients as a part of standard medical care. For these treatments only dose regimen information must be recorded into e-CRF and source documentation.

5.8 Concomitant medications

5.8.1 Permitted medications

Permitted medications and anti-infective prophylaxis can be carried out in accordance with the local standards of the participating centers. In the absence of local standards, it is possible to use the following scheme:

- Infusion therapy in a volume of 3 l/m^2 from D-7 to D+5, then 30 ml/kg/day before engraftment and transfer to oral hydration.

- Oral hydration of at least 30 ml/kg/day after stopping infusion therapy and until immunosuppressive therapy is discontinued.
- Ondansetron 8 mg TID i.v. on days of taking busulfan and cyclophosphamide and for at least 1 day after. With insufficient effect or history of severe nausea after chemotherapy - aprepitant 125-80-80 mg.
- Levetiracetam 1000 mg 2 times a day per os, starting 24 hours before the first dose of busulfan, 24 hours after the last dose of busulfan. Relanium jet or microjet with the development of neurotoxicity.
- Allopurinol 300 mg 1 time per day from D-7 to D + 5
- Omeprazole 20-40 mg 2 times a day from D-7 to D + 180
- Sulfamethoxazole / trimethoprim 960 mg 1 time per day from D-7 to D + 180
- Acyclovir 200 mg 3 times a day from D-7 to D + 180
- Fluconazole 400 mg 1 time per day from D0 until engraftment. In the presence of probable or proven invasive aspergillosis, voriconazole 200 mg BID from D0. It is advisable to avoid the use of voriconazole during conditioning. If active treatment of invasive aspergillosis is necessary, it is recommended one day before the first administration of busulfan to transfer the patient to echinocandin and resume therapy with voriconazole 24 after the last administration of busulfan. Alternatively, posaconazole 200 mg TID or 400 mg BID can be used.
- Ciprofloxacin 200 mg BID i.v. from D0 until engraftment, then 500 mg BID per os to D + 60. Further, amoxicillin 500 mg TID from D + 61 to D + 180.
- For women, 0.03 mg Ethinylestradiol 0.150 mg Levonorgestrel 1 tablet 1 time per day from D-7 to platelet levels above 50 thousand per μl after engraftment OR buserelin nasal 0.15 mg TID from D-7 to a platelet level of more than 50 thousand per μl after engraftment OR buserelin depot 3.75 mg every 4 weeks subcutaneously OR goserelin 10.8 mg once before transplantation.

5.8.2 Prohibited medications

During the study period the patient should not receive any kinase inhibitors but ruxolitinib. The only exception is post-transplant relapse prophylaxis in Ph-positive ALL patients if that is a part of local center's standard practice.

The following registered drugs affecting the immune system should also be avoided: anti-human T-lymphocyte immunoglobulin, tumor necrosis factor antagonists, antagonists of interleukin-6 or its receptor, anti-CD19, CD25, or CD56 antibodies.

The patient should not receive immune checkpoints inhibitors, e.g. nivolumab, pembrolizumab, avelumab etc.

6 EFFICACY AND SAFETY ASSESMENTS

6.1 Efficacy assesments

The presence of acute GVHD is confirmed either by morphology or based on complex evaluation performed by a head of appropriate study center clinical unit. The morphological confirmation of acute gut GVHD involving intestine or gaster is mandatory. In patients with skin or hepatic acute GVHD the clinical diagnosis is also appropriate.

The types of acute GVHD and stage of GVHD and response to steroids are determined by treating physician according to international classification (MAGIC criteria, Harris et al., 2016) and presented in Table 6-1. Since acute and chronic GVHD is one of the endpoints, the physicians will follow the discrimination between these two forms based on MAGIC definition also presented in Table 6-1.

Table 6-1: Acute GVHD types and staging

Acute GvHD onset		
Classic	First episode of aGvHD*	≤Day 100
Late	First episode of aGvHD*	>Day 100
Recurrent	Recurrence of aGvHD*, after a period of aGvHD control, inactivity or resolution	>Day 100
Persistent	aGvHD* signs persist beyond day 100 from a prior active classic aGvHD	>Day 100

*Presenting acute features only: maculopapular erythematous skin rash; and/or hyperbilirubinemia; and/or anorexia with weight loss, nausea, vomiting, diarrhea, severe abdominal pain, GI bleeding and/or ileus

Stage	**Skin**	**Liver (bilirubin)**	**Stomach**	**Intestine (stool volume)**
0	No active (erythematous) GVHD rash	<35 mkmol/l	No or intermittent nausea, vomiting, or anorexia	<500 ml/day
1	Maculopapular rash <25% of body surface	35-50 mkmol/l	Persistent nausea, vomiting or anorexia	500-1000 ml/day
2	Maculopapular rash 25-50% of body surface	51-100 mkmol/l		1000-1500 ml/day
3	Maculopapular rash >50% of body surface	101-260 mkmol/l		>1500 ml/day
4	Generalized erythroderma (>50%	>260 mkmol/l		Intensive abdominal pain

	of body surface) plus bullous formation and desquamation >5% of body surface			or ileus, intestinal bleeding
Overall stage				
I	Stages 1-2	No	No	No
II	Stage 3 or	Stage 1 or	Stage 1 or	Stage 1
III	-	Stage 2-3 or	Stage 0-1	Stage 2-3
IV	Stage 4 or	Stage 4	Stage 0-1	Stage 4

Presence of chronic GVHD is evaluated based on clinical signs or morphological picture. However, histological confirmation is not required for chronic GVHD diagnosis. The final diagnosis may be based on evaluation of clinical signs by the head of a transplant unit in one of sites.

The chronic GVHD severity is assessed according to NIH 2015 consensus (Jagasia et al., 2015) with separate overall grade and organ involvement grade evaluation (Jagasia et al., 2015) presented in Table 6-2.

Table 6-2: Overall grade and organ involvement grade evaluation

Grade	**0**	**1**	**2**	**3**
General functions	No symptoms	Some symptoms present, outpatient treatment, some functional limitations to physical activity (ECOG 1, Karnofsky 80-90%)	Some symptoms present, outpatient treatment, the patient is capable of self-service, >50% time spent actively (ECOG 2, Karnofsky 60-70%)	Some symptoms present, outpatient treatment, limited capability for self-service, >50% of time spent in bed (ECOG 3-5, Karnofsky <60%)
Skin	No symptoms	<18% of body surface involved, no sclerotic changes	19-50% of body surface involved or superficial sclerotic changes (the	>50% of body surface involved or deep sclerotic changes (no skin fold can be

Grade	0	1	2	3
			skin fold can be formed)	formed) or limited mobility, ulceration or intensive itching
Oropharynx mucosa	No symptoms	Mild symptoms, normal food intake	Moderate symptoms severity with partial limitation of food intake	Severe symptoms with significant oral food intake limitation
Eyes	No symptoms	Mild sicca syndrome symptoms, normal everyday activity (requires moisturizing ≤3 times\day) or asymptomic stream keratoconjunctivitis sicca	Moderate sicca syndrome symptoms, partial everyday activity limitation (requires moisturizing ≥3 times\day) without eyesight impairment	Severe sicca syndrome symptoms, significant everyday activity limitation (requires analgesia) or inability to work due to ophthalmological symptoms or loss of vision due to keratoconjunctivitis Sicca
Gastrointestinal tract	No symptoms	Dysphagy, anorexy, nausea, vomiting, gastric pain or diarrhea without significant weight loss (<5%).	Symptomatic with moderate weight loss (5-15%)	Symptomatic with significant weight loss (>15%) requiring nutritional support or esophageal surgery
Liver	Normal biochemistry values with PA, ALAT or ASAT	Normal bilirubin, ALAT or ASAT ≥3 norms and <5	Raised total bilirubin, but less than 50 mkmol/l or	Bilirubin >50 mkmol/l

Grade	0	1	2	3
	< 3 norms	norms or AP ≥3 norms	ALAT ≥5 norms	
Lungs	No symptoms FEV1>80% or LFS (Lung function score)=2	Mild symptoms (dyspnea after climbing 1 floor of stairs) FEV1 60-79% or LFS 3-5	Moderate symptoms (dyspnea after walking on square surface) FEV1 40-59% or LFS 6-9	Severe symptoms (dyspnea in rest, O2 dependence) FEV1<59% or LFS 10-12
LFS is calculated as a sum of score points for FEV1 and diffusion capacity: >80%= 1; 70-79%= 2; 60-69%=3; 50-59% = 4; 40-49% = 5; <40% = 6. Diffusion capacity is evaluated based on hematocrit = diffusion capacity measurement + (hematocrit -44)* 1.35%.				
Joints and fasciae	No symptoms	Mild discomfort while moving arms and legs, normal or slightly decreased movement range, no impairment to everyday activity	Discomfort while moving arms and legs or joint contractures, erythema due to fasciitis, moderate movement range decrease and mild to moderate impairment to everyday activity	Contractures with severe decrease of movement range and severe impairment to everyday activity (inability to tie shoelaces, button up a coat, undress without help etc.)
Genitalia	No symptoms	Some symptoms with mild visual changes with no discomfort during coitus or gynecological examination	Some symptoms accompanied by moderate visual changes with no tenderness during coitus or gynecological examination	Some symptoms accompanied by evident visual changes (strictures, pudendal lips agglutination or severe ulceration) accompanied by pronounced tenderness

Grade	0	1	2	3
				during coitus or gynecological examination
Other signs of GVHD: Ascitis Polyserositis Hydropericardium nephrotic syndrome Perypheral neuropathy Polymyositis Eosinophilia more than 500 per µl.	No symptoms	Mild symptoms	Moderate symptoms	Severe form
Overall chronic GVHD grade (if applicable)		Grade 0: no GVHD symptoms I (mild): 1-2 organs involvement with maximal grade 1 (besides lungs). II (moderate severity): at least 1 organ with changes grade ≥2 or ≥3 organs with maximal grade 1 or lungs with grade ≥1 III (severe): at least 1 organ with grade ≥3 or lungs with grade ≥2		

Overall survival (OS), acute GVHD, chronic GVHD, time to relapse, transplant-related mortality were evaluated as time from transplant to event.

Relapse is diagnosed in patients with more than 5% of clonal blast cells at the time of HSCT or less than 5% blast cells with pre-transplant phenotype after any specific antitumor therapy was administered (donor lymphocyte infusion, chemotherapy, targeted therapy). During event-free survival (EFS) evaluations events re death, relapse or primary non-engraftment. For relapse-free survival (RFS) or GVHD-free survival evaluation events were death due to any reason, relapse, Gr III-IV acute GVHD, moderate or severe chroniv GVHD (Hotlan et al., 2015).

The primary non-engraftment is defined as lack of donor chimerism in bone marrow aspirate at Д+40. Donor chimerism determination after HSCT requires recipient and donor genetic profile determination prior to HSCT with subsequent evaluation of ratio of donor-derived and recipient-derived cells in recipient's peripheral blood and bone marrow. The method of chimerism determination is not limited by current protocol. The study center may use any effective method, which corresponds to its standard operation procedures. Time to engraftment is evaluated as time

from transplant to the moment of reaching peripheral neutrophil count of more than 500 cells per µl without G-CSF stimulation.

6.2 Safety assessments

The frequency and selection of instrumental studies and laboratory tests used to evaluate the safety in this study is determined by standard operational procedures of participating centers. However in all patients chimerism should be evaluated at least once, at D+100 after HSCT.

The clinical complications of hemopoietic stem cell transplant procedure are evaluated according to the following criteria:

- Sepsis is diagnosed based on Sepsis-3 (Singer et al., 2016) criteria OR presence of positive blood culture obtained during episode of fever;
- Severe sepsis is diagnosed in a patient fulfilling sepsis criteria and with signs of organ failure;
- Invasive mycosis is diagnosed based on invasive mycoses diagnostics and treatment criteria ECIL-4 (Groll et al., 2014);
- Hepatic veno-occlusive disease is diagnosed and staged according to revised EBMT-2016 criteria (Mohty et al., 2016);
- Thrombotic microangiopathy is diagnosed based on "General TMA" criteria (Cho et al., 2010);
- CMV reactivation is determined based on quantative real-time PCR data and NGS results. The lower copy number threshold for CMV reactivation is 500 copies per ml. Any infection (with lungs, GIT or bone marrow involvement) is treated as reactivation even if there are no positive blood tests;
- All other complication during safety evaluation are determined and staged according to NCI CTCAE 5.0.

6.3 Adverse events

6.3.1 Definitions

An adverse event is defined as the appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s), or medical condition(s) that occur after patient's signed informed consent has been obtained.

Abnormal laboratory values or test results occurring after informed consent constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, require therapy (e.g., hematologic abnormality that requires transfusion or hematological stem cell support), or require changes in study medication(s).

Adverse events considered related by the investigator to the investigational treatment that begin or worsen after informed consent should be recorded in the Adverse Events CRF. Conditions that were already present at the time of informed consent should be recorded in the Medical History page of the patient's CRF. Adverse event monitoring should be continued for at least 30 days following the last dose of study treatment. Adverse events (including lab abnormalities that constitute AEs) should be described using a diagnosis whenever possible, rather than individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate Adverse Event.

Adverse events will be assessed and graded according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

If CTCAE grading does not exist for an adverse event, the severity of mild, moderate, severe, and life-threatening, death related to the AE corresponding respectively to Grades 1 - 5, will be used.

The occurrence of adverse events should be sought by non-directive questioning of the patient (subject) during the screening process after signing informed consent and at each visit during the study. Adverse events also may be detected when they are volunteered by the patient (subject) during the screening process or between visits, or through physical examination, laboratory test, or other assessments. As far as possible, each adverse event should be evaluated to determine:

- The severity grade (CTCAE Grade 1-5);
- Its duration (Start and end dates);
- Its relationship to the study treatment;
- Action taken with respect to study treatment;
- Whether medication or therapy was given (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy);
- Whether it is serious, where a serious adverse event (SAE) and which seriousness criteria have been met.

6.3.2 Seriousness criteria

Serious adverse event (SAE) is defined as one of the following:

- Is fatal or life-threatening;
- Results in persistent or significant disability/incapacity;
- Constitutes a congenital anomaly/birth defect;
- Is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above;

- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Note that hospitalizations for the following reasons should not be reported as serious adverse events:
- Routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
- Elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
- Social reasons and respite care in the absence of any deterioration in the patient's general condition
- Note that treatment on an emergency outpatient basis that does not result in hospital admission and involves an event not fulfilling any of the definitions of a SAE given above is not a serious adverse event
- Note that progression of malignancy (including fatal outcomes), if documented by use of appropriate method (as per 2014 NIH response criteria, Lee 2015), should not be reported as a serious adverse event.

6.3.3 Reporting

To ensure patient safety, every SAE considered related to investigational treatment by the investigator occurring after the patient has provided main informed consent and until at least 30 days after the patient has stopped study treatment must be reported to the study sponsor within 24 hours of learning of its occurrence.

Any additional information for the SAE including complications, progression of the initial SAE, and recurrent episodes must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Any SAEs experienced after the 30 day safety evaluation follow-up period should only be reported to the study sponsor if the investigator suspects a causal relationship to the study treatment.

Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form; all applicable sections of the form must be completed in order to provide a clinically thorough report. The investigator must assess and record the relationship of each SAE to each specific study treatment (if there is more than one study treatment), complete the SAE Report Form in English, and submit the completed form within 24 hours to the study sponsor. Detailed instructions regarding the SAE submission process and requirements for signatures are to be found in the investigator folder provided to each site.

Follow-up information is submitted in the same way as the original SAE Report. Each reoccurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the patient continued or withdrew from study participation. If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to the study treatment, the study sponsor may urgently require further information from the investigator for Health Authority reporting.

The sponsor may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported.

The study sponsor will collect the following safety information:

- All SAEs;
- All records of drug exposure during pregnancy;
- All non-serious AEs;
- All reports of misuse and abuse of an IMP, other medication errors and uses outside of what is foreseen in the protocol (irrespective if a clinical event has occurred).

As soon as Novartis is a marketing authorization holder of IMP, the following safety information must be transferred to Novartis safety department by study sponsor within 15 days of awareness:

- All collected SAEs in subjects exposed to the Novartis IMP;
- All collected pregnancy reports in subjects exposed to the Novartis IMP;
- All collected reports of abuse and misuse of the Novartis IMP.

6.3.4 Pregnancy

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to the study sponsor within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the study sponsor. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

Pregnancy outcomes must be collected for the female partners of any males randomized to BAT who took study treatment in this study (if required per label or as per local regulation). Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

7 DATA ANALYSIS AND STATISTICAL METHODS

The size of the patient group is calculated based on the primary objective of the study. In the pilot study in patients with myelofibrosis, the incidence of acute GVHD II-IV with the use of PTCy-ruxolitinib prophylaxis was 25%. According to the literature, the incidence of acute GVHD with the use of the PTCy-CNI-MMF combination is 15–20% (Luznik L, 2008, and others). For analysis, a value of 20% was taken. The Table 7-1 below shows the size of the sample, options for the boundaries of non-inferiority, significance and power of the study.

Table 7-1: Sample size

Sample size	N in each group	GVHD rate in control group	Study power	p-value	Non-inferiority border
504	252	20%	80%	0.025	10%
224	112	20%	80%	0.025	15%
128	64	20%	80%	0.025	20%
396	198	20%	80%	0.05	10%
176	88	20%	80%	0.05	15%
98	49	20%	80%	0.05	20%
2188	1094	20%	80%	0.025	10%
548	274	20%	80%	0.025	15%
244	122	20%	80%	0.025	20%
1724	862	20%	80%	0.05	10%
432	216	20%	80%	0.05	15%
192	96	20%	80%	0.05	20%

With a double margin of significance, the margin of difference is 20% and the power of the study is 80%, the size of the group was 64 people in each group.

Stratification during randomization will include 2 parameters that most significantly affect the results of HSCT with PTCy: the disease risk index (DRI, Armand P, 2014) and the type of the donor (unrelated or haploidentical). Given that one of the secondary points of the study is the frequency of relapses, using the risk index of the disease will allow the group to balance this risk and reliably evaluate the effect of the prophylaxis regimen on this parameter. The use of stratification by type of the donor is based on the registry studies that demonstrated that despite comparable efficacy of haploidentical transplantation with MRD and MUD after conventional GVHD prophylaxis, when compared to PTCy-based MUD, haploidetical demonstrate worse outcome primary due to graft failure and higher incidence of GVHD (Ruggeri A et al., 2018,

Brissot E et al. Haematologica. 2015). Given that the primary objective of the study is to compare the frequency of acute GVHD, a correction is necessary for this factor.

The distribution among the test arms will be based on the chi-square test. The goal in the distribution will be to achieve the maximum sum of alpha values for DRI and type of donor.

Comparison of cumulative frequencies in the study groups is planned using the Gray test. Comparison of survival rates is planned using the Kaplan-Meier method and log-rank test. Comparison of complication rates is planned using the Fisher and chi-square tests. To assess the non-inferiority it is planned to use the Faring Manning test. Equivalence will be ascertained by insignificant results of both non-inferioty and superiority tests. The significance level for all tests is set at 0.05.

Intermediate analyzes of the study will be conducted annually after the inclusion into the study of 40 and 80 patients. The interim analysis will assess the following parameters: overall survival, event-free survival (event: relapse, death and primary graft failure), cumulative relapse rate, non-relapse mortality, acute GVHD II-IV degree, moderate and severe chronic GVHD (according to the NIH 2018 criteria), the incidence of severe poor graft function (leukopenia, neutropenia or thrombocytopenia, grade 4).

8 DIRECT ACCESS TO SOURCE DATA

The investigator agrees to keep the following study-related records during 15 years after the end of the study:

- Site specific Investigator's trial master file;
- All original signed informed consent forms and information sheets;
- All filled case report forms;
- All SAE reports;
- Relevant correspondence;
- Source documents

The investigators agrees to provide all mentioned above documentation available for regulatory authorities or sponsor’s audits. If the investigators becomes unable to continue to retain study-related records, he must to notify the sponsor.

9 AUDITS AND INSPECTIONS / QUALITY CONTROL

Authorized representatives of sponsor or a regulatory authority may perform audits or inspections at the investigational sites, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded,

analyzed, and accurately reported according to the protocol, GCP and any applicable regulatory requirements. The investigator will contact sponsor immediately if contacted by a regulatory agency about an inspection at the site.

10 ETHICAL CONSIDERATIONS

10.1 Institutional review board / Independent ethics committee

The study protocol, protocol amendments, patient information sheet and informed consent form, case report form and other relevant documents will approved in Central Ethics Committee (CEC) of Russian MoH and local Ethics Committees (LEC) in each investigational site. Submission the mentioned above documentation to the CEC is under the sponsor's responsibility, submission the documents to LEC is under the investigator's responsibility. All correspondence with CEC/LEC (e.g. notification letters, submission letters, approval letters, CEC/LEC SOPs, etc.) will be retained in the appropriate section of the General trial master file.

10.2 Patient information and informed consent

The investigator, or a person designated by the investigator, will obtain written informed consent from each patient before the start of any study-related procedures. The investigator must ensure that each patient is fully informed about the objectives of the study and possible risks/benefits associated with participation.

10.3 Confidentiality

All parties will ensure protection of subject personal data and will not include subject names on any sponsor forms, reports, publications, or in any other disclosures, except where required by laws.

Patient personal data will be replaced by a numerical code consisting order to de-identify the trial subject.

10.4 Study discontinuation

Each investigational site will be closed during the site closure-visit after the LPLV.

The end of the clinical part of the study is defined as the time at which it is deemed that sufficient subjects have been recruited and completed the study as stated in the protocol.

The end of the whole study is defined as the moment, when the CSR is finalized.

An investigator in any centre has the right for its own publication and presentation of study results at conferences (congresses, symposia, etc.) providing that a written agreement regarding the fact, place and content of the publication from the sponsor's representative and providing that all data for publication have been obtained in its center.

Writing of research works that are based on the results of the study is allowed as long as there is preliminary written agreement with the sponsor.

10.5 Confidentiality

All parties will ensure protection of subject personal data and will not include subject names on any sponsor forms, reports, publications, or in any other disclosures, except where required by laws.

Patient personal data will be replaced by a numerical code consisting order to de-identify the trial subject.

11 DATA MANAGEMENT

In this study will be used an electronic case report form (e-CRF)

e-CRF does not contain fields for data recorded directly and considered as primary data (without prior written or electronic recording); All data entered in the e-CRF must be indicated in the sourse documentation.

The investigator is fully responsible for the collection and provision of all clinical and laboratory data, as well as safety data that are entered into the e-CRF, and must guarantee their authenticity and compliance with the primary documentation.

If necessary, after the completion of the clinical part of the study, the sponsor may request the investigator to provide an explanation for any entry in the e-cRF by sending a special data data resolution form.

12 AUDITS AND INSPECTIONS / QUALITY CONTROL

Authorized representatives of sponsor or a regulatory authority may perform audits or inspections at the investigational sites, including source data verification. The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, GCP and any applicable regulatory requirements. The investigator will contact sponsor immediately if contacted by a regulatory agency about an inspection at the site.

13 FINANCIAL ASPECTS AND INSURANCE

The life and health of all patients who signed the ICF form and successfully completed all screening procedures will be insured in accordance with the RF PP No. 714 “On the approval of standard rules for compulsory life and health insurance of a patient participating in clinical trials of a medicinal product”. The sponsor is responsible for the insurance of study subjects.

The financial aspects of the study will be described in separate agreements.

14 STUDY RESULTS AND PUBLICATIONS

Results of this study can be published or made public in any other manner by an investigator only way by a written assent given by an authorized sponsor’s representative. Among authors of

a centralized publication should be listed principal investigators from all centres. It is possible to include as co-authors co-investigators, representatives of the sponsor company or the organization that is responsible for conducting this study.

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16 APPENDICES

16.1 Appendix 1: Dose adjustment of calcineurin inhibitors

The chart for Cyclosporine A dose correction depending on its concentration:

- 100-150 ng/ml: the dose is increased by 25%
- 50-100 ng/ml: the dose is increased by 50-75%
- 350-500 ng/ml: the dose is increased by 25-50%
- 500-1000 ng/ml: pause in infusion or oral intake for 24 hours, dose reduction by 50%
- >1000 ng/ml: pause in infusion or oral intake for 48 hours, dose reduction by 50%, before the drug is reinitiated a new concentration test is highly advisable

The shift to oral cyclosporine A is performed after engraftment if the patient has no severe mucositis impairing oral intake of the drug. The recommended coefficient followed when shifting from iv to oral cyclosporine A forms based on its blood concentration:

- 300-350 ng/ml: 1:1
- 200-300 ng/ml: 1:1.3
- 150-200 ng/ml: 1:1.5
- <150 ng/ml: 1:1.8

During the shift it is important to provide adequate hydration (oral or intravenous).

The drug is taken up to the D+100, then tapered by 25% of initial dose once per week (withdrawed in 8 weeks).

The chart for tacrolimus dose correction depending on its concentration:

- 2-3 ng/ml: the dose is increased by 25%
- <2 ng/ml: the dose is increased by 50-75%
- 12-18 ng/ml: the dose is increased by 25-50%
- 18-30 ng/ml: pause in infusion or oral intake for 24 hours, dose reduction by 50%
- >30 ng/ml: pause in infusion or oral intake for 48 hours, dose reduction by 50%, before the drug is reinitiated a new concentration test is highly advisable.

The shift to oral tacrolimus is performed after engraftment if the patient has no severe mucositis impairing oral intake of the drug. The recommended coefficient followed when shifting from iv to oral tacrolimus forms based on its blood concentration:

- 10-15 ng/ml: 1:1
- 7-10 ng/ml: 1:1.3
- 5-7 ng/ml: 1:1.5
- <5 ng/ml: 1:1.8

During the shift it is important to provide adequate hydration (oral or intravenous).

The drug is taken up to the D+100, then tapered by 25% of initial dose once per week (withdrawed in 8 weeks).

If acute renal damage develops the preemptive CNIs dose reduction by 25-50% is recommended as in patients with inadequate renal function the drug is rapidly accumulated, which may lead to further renal damage.

16.2 Appendix 2: Clinical manifestations of the cytokine release syndrome and clinical intervention

Clinical signs	Grade 1	Grade 2	Grade 3	Grade 4
Fever	Yes	Yes	Yes	Yes
Hypotension	No	Response to cristalloids and low vasopressor doses	High vasopressor doses	Poor response to vasopressors, life-threatening
Respiratory failure	No	ALV not required, oxygen inhalation	Fast-flow noninvasive ventilation or significant oxygen concentrations required	ALV required
Sinus tachycardia	Asymptomatic	Symptomatic, no correction required	Symptomatic, urgent correction required	_
Arrhythmia	Asymptomatic	Symptomatic, no correction required	Symptomatic, urgent correction required	Life-threatening
Low ejection fraction	No	EF 40-50% or 10-19% decrease compared to baseline	EF 20-39% or >20% decrease compared to baseline	EF<20%
Pleural effusion	Asymptomatic	Symptomatic, requires diuretics or thoracocentesis	Symptomatic, with respiratory failure	-
Pulmonary edema	Minimal dyspnea	Moderate dyspnea, decrease in everyday activity	Dyspnea at rest, oxygenation required	ALV required
Vomiting	1-2 episodes	3-5 episodes	More than 6 episodes	Life-threatening
Diarrhea	1-3 times daily	4-6 times daily	>6 times daily	Life-threatening
Raised ALAT/ASAT	1-3 norms	3-5 norms	5-20 norms	>20 norms
Raised bilirubin	1-1.5 norms	1.5-3 norms	3-10 norms	>10 norms

Clinical signs	Grade 1	Grade 2	Grade 3	Grade 4
Diuresis	-	-	oliguria	anuria
ARF, creatinin	1.5-2 norms	2-3 norms	>3 norms	Dialysis required
DIC	-	Only laboratory changes	Laboratory changes and hemorrhagic syndrome	Life-threatening
Rash	<10% of body surface area	10-30% of body surface area	>30% of body surface area	Life-threatening
Medical tactics	Symptomatic therapy until PTCy	Symptomatic therapy until PTCy	Adequate supportive therapy depending on clinical signs, transfer to ICU recommended, specific therapy	Transfer to ICU required, specific therapy should be given in first 24 hours
Tocilizumab 8 mg/kg	-	-	+	+
Steroids 1-2 mg/kg	-	-	+	+
Ruxolitinib15-20 mg/day	-	-	-	+

The CRS most often develops in first hours after transplant infusion. Several SRC symptoms are usually present, severe forms may be presented by multiorgan failure. The most difficult differentional diagnosis is with hepatic veno-occlusive disease and severe sepsis. As this protocol is aimed at recruitment of patients in remission, they are not likely to have agranulocytosis at D0. Also, veno-occlusive disease more often develops at egraftment, early cases are seen in about 10% of all cases. Therefore for this protocol all cases of multiorgan failure at D0-D+1 should be viewed as CRS.