https://doi.org/10.23873/2074-0506-2022-14-4-462-475

Regulatory T cells and allogeneic hematopoietic stem cell transplantation

O.S. Karavaeva✉, M.Yu. Drokov, E.G. Khamaganova

National Medical Research Center for Hematology,

4 Noviy Zykovskiy Dr., Moscow 125167 Russia

✉Corresponding author: Olga S. Karavaeva, Postgraduate, Hematologist, National Medical Research Center for Hematology, olga.starikova.1994@mail.ru

Abstract

The article has summarized the available data about regulatory T cells, describing in-detail the stages of their studying, their development, classification, mechanisms of immunosuppression in general terms and also in the context of allogeneic hematopoietic stem cells transplantation. The effect of immunosuppressive agents on this cell population is considered.

The role of regulatory T cells in the pathogenesis of both acute and chronic graft-versus-host disease has been revealed. The possibilities of clinical use of regulatory T cells (including modified regulatory T cells) in the prevention and treatment of these complications are described in detail.

Keywords: regulatory T cells, Treg, allogeneic hematopoietic stem cell transplantation, acute graft-versus-host disease, chronic graft-versus-host disease

Conflict of interests. Authors declare no conflict of interest.

Financing. The study was performed without external funding.

Acknowledgments The team of authors expresses their gratitude to N.N. Popova, the Researcher of the Sector for Scientific Research in Chemoblastosis Chemotherapy, Hematopoietic Depressions, and Bone Marrow Transplantation of the National Medical Research Center for Hematology of the Ministry of Health of the Russian Federation for her contributing help in preparing this article.

For citation: Karavaeva OS, Drokov MYu, Khamaganova EG. Regulatory T-cells and allogeneic hematopoietic stem cell transplantation. *Transplantologiya. The Russian Journal of Transplantation.* 2022;14(4):462–475. (In Russ.). https://doi.org/10.23873/2074-0506-2022- 14-4-462-475

aGVHD, acute graft-versus-host disease

allo-HSCT, allogeneic hematopoietic stem cell transplantation

APC, antigen presenting cells

ATG, antithymocyte globulin

CAR, chimeric antigen receptor

cGVHD, chronic graft-versus-host disease

DN, double negative thymocytes

DP, double positive thymocytes

FoxP3, FoxP3-negative

GCS, glucocorticosteroids

GVHD, graft-versus-host disease

GVT, graft-versus-tumor effect/activity

HLA, human leukocyte antigen

IL, interleukin

iTregs, induced Tregs

MHC, major histocompatibility complex

MPM, mycophenolate mofetil

mTOR, mammalian target of rapamycin

pTregs, peripheral Tregs

SP, single positive

TCR, T-cell receptor

TGFβ, transforming growth factor β

Treg(s), regulatory T cell(s)

tTregs, thymic or natural Tregs

Regulatory T cells: history of discovery, function

Regulatory T cells (Tregs) constitute a subset of T lymphocytes that provide peripheral tolerance by controlling the power and duration of the immune response through the regulation of the T-effector function [1].

Treg research began more than half a century ago. So, in 1971, an article was published, which suggested the existence of a population of suppressor lymphocytes of thymic origin, capable of reducing the anti-infective response of T lymphocytes [2]. Over the next 10 years, many papers were published demonstrating the inhibitory properties of T-suppressor cells. However, in the early 80s, with the development of molecular biology, the research data in this area were called into question, and the term "T suppressors" almost disappeared.

The “second” life was given to studies of this cell population after the publication of reports on the development of autoimmune complications in mice subjected to thymectomy [3], as well as on the positive effect of infusing T lymphocytes of a certain phenotype on the autoimmune process [4]. Thus, the term "T-suppressor cells" was transformed into Tregs, due to their ability to "regulate" the immune response.

For a long time, the search for membrane markers for more accurate isolation of this cell population was carried out, and in the early 2000s, approximately simultaneously, two facts were discovered: a) the Treg population is functionally dependent on interleukin-2 (IL-2); b) it is characterized by a high expression of receptors for the cytokine CD 25 [5] , and also that this cell population is characterized by the presence of the transcription factor FoxP3 [6]. Subsequently, a negative correlation was demonstrated between the expression of FoxP3 and the expression of

the CD 127 receptor for IL-7 [7]. Thus, to date, in most studies, the Treg population is distinguished as follows: CD3+ CD4+ $CD25^{hi}$ $CD127^{low}$.

It is important to mention that there are other populations of lymphocytes with regulatory potential. In particular, B-regulatory cells [8], which realize their immunosuppressive properties through direct intercellular interaction and through the secretion of IL-10; as well as the CD8+ Treg population, which acts mainly due to cytolysis (with the participation of perforin, granzyme A and B) [9]. However, in this article we will focus on CD4+ Tregs.

As already mentioned, the role of Tregs in the prevention of autoimmune reactions has been proven. [10], as well as in the induction of tolerance to the allograft [11], a decrease in antitumor immunity - an increase in the number of Tregs in the tumor microenvironment has been described [12].

Regulatory T cells: stages of maturation, and classification

By origin, Tregs are divided into thymic or natural Tregs (tTregs), and induced or peripheral Tregs (iTregs, pTregs) [13]. It is important to note that the second group of cells is called "induced" when the transformation into Tregs occurred in vitro, and "peripheral" if it did in vivo.

To understand the formation of tTregs, it is necessary to refer to the stages of T-lymphocyte differentiation. The development of T-lymphocytes begins in the bone marrow, where an early T-cell precursor is formed from the precursor cell of lymphopoiesis, which migrates into the thymus, namely into its cortical substance [14]. These earliest thymocytes lack CD4 and CD8 expression and are therefore referred to as double negative (DN) cells. About 5% of these cells acquire the γδ-T-cell receptor (TCR) and, without passing through “positive” and “negative”

selections, leave the thymus entering the peripheral blood, where they implement the function of the primary immune response.

The majority of DN thymocytes follow the path of formation of αβ-TCR: these cells will later become T-helpers (CD4+) and T-cytotoxic lymphocytes (CD 8+). At the first stage, DN undergo TCR rearrangement with the formation of CD4+CD8+ double positive thymocytes (DP).

The next stage is called "positive selection". At this stage, the TCR of DP thymocytes is checked for the ability to bind to the molecule of the major histocompatibility complex class I (MHC-I) or class II (MHC-II); meanwhile one of the receptors ceases to be expressed on the membrane and the CD4+ or CD8+ single positive (SP) thymocytes are formed. Cells that have created non-functional TCR are subjected to apoptosis.

Further, SP thymocytes migrate to the thymus medulla where the stage of "negative selection" occurs, in which autoreactive T lymphocytes, namely the thymocytes with a high degree of TCR affinity for MHC, undergo apoptosis [15]. Interestingly, some of these thymocytes do not undergo negative selection, but differentiate into Tregs [16]. It is important to note that the TCR repertoire of human tTregs overlaps only by a quarter with the repertoire of other, “non-regulatory” CD4+ cells [17].

The question is widely discussed: at what stage of differentiation the thymocyte acquires adherence to the Treg lineage [18]. Thus, the population expressing CD25 and FoxP3 is mainly represented by CD4+ SP thymocytes, with a small proportion of CD8+ SP [16], DP, and DN cells.

Cells that have passed all stages acquire the CD45RA+CCR7+ phenotype, leave the thymus entering the peripheral blood, where they are called naive T lymphocytes (rather than thymocytes).

Peripheral Tregs are formed of naive (non-regulatory) CD4+ FoxP3-negative (FoxP3-) cells in the periphery under the influence of transforming growth factor β (TGFβ) and IL-2 [19]. Peripheral Tregs are designed to regulate the immune response to “non-autoantigens,” such as food antigens, gut microbiota antigens, and fetal antigens [20]. This fact is confirmed by the change in the TCR repertoire of gut Tregs after antibiotic therapy [21]. However, it is important to note that not only pTregs, but also tTregs perform the regulatory function in the gut, which was demonstrated in the paper by D. F. Zegarra-Ruiz et al. Gut microbiota antigens are represented as T cells in the thymus [22] .

It should be noted that, despite the fundamentally different sources of origin, tTregs and pTregs (iTregs) cannot be distinguished by membrane markers. To date, it is possible to differentiate one population from another using the analysis of epigenetic structures, namely the locus encoding FoxP3 [13]. The stability of FoxP3 expression also varies: pTregs (iTregs) under inflammatory conditions can differentiate into effector cells [23].

As noted above, the TCR repertoire differs between the population of tTregs and "non-regulatory" CD4+ cells [17]. Taking into account that pTregs are formed of “non-regulatory” T-helper populations, this feature can also be used to divide subsets of tTregs and pTregs: the tTreg TCR repertoire is prone to the recognition of auto-antigens, and that of the pTreg TCR is aimed more at recognition of alien antigens [24].

It is also possible to classify Tregs by maturity (like other T cells) based on the expression of CD45RA, CD62L, and CCR7 (CD197). Thus, it is possible to distinguish naive cells, central memory T cells, effector memory T cells, and effector Tregs [25]. It should be noted that the ratio of these Treg subpopulations changes with age: normally, in young people, about 30% of Tregs are naive [26], and later, due to the involution

of the thymus, the proportion of naive Tregs decreases, giving way to memory Tregs [27].

Immunosuppression mechanisms of regulatory T cells

The mechanisms by which Tregs suppress the immune response are extremely diverse. According to the type of effect, they can be distributed into four groups: cytolysis, secretion of inhibitory cytokines, effects on dendritic cells, and metabolic disorders [1].

Tregs were long thought to have no cytolytic activity, but a 2006 study demonstrated that Tregs can kill B-cells in a granzyme B-dependent and, in part, perforin-dependent manner [28]. In another study, cytolysis of Tregs in the tumor microenvironment was shown to kill cytotoxic T lymphocytes and NK cells, thereby reducing the antitumor response [29].

In our earlier study [30], we demonstrated that in patients undergoing allogeneic hematopoietic stem cell transplantation (alloHSCT), a low amount of granzyme B-positive Tregs determined a high likelihood of developing an acute graft-versus-host disease (aGVHD); the minimum number of these cells was determined on the day of establishing aGVHD diagnosis. On the contrary, the probability of aGVHD decreased with an increase in the number of granzyme B-positive Tregs.

Treg-produced inhibitory cytokines include IL-10, TGFβ, IL-35. These cytokines suppress the activation and proliferation of effector T and B lymphocytes, and can also induce the formation of pTregs and B-regulatory cells from non-regulatory populations [31]. The immunosuppressive properties of IL-10 have been demonstrated, for example, in mouse models of inflammatory bowel disease [32], and in suppression of allergic inflammation in bronchial asthma [33]. The role

of both IL-10 and TGFβ has been studied in the suppression of the antitumor response in the tumor microenvironment [34].

Interestingly, TGFβ performs a suppressive function not only as a Treg-secreted cytokine, but also through intercellular interaction in the form associated with the Treg membrane. This has been demonstrated, for example, in a model of type I diabetes mellitus, where due to the effect of TGFβ associated with the Treg membrane, the infiltration of CD8+ by lymphocytes of the islets of Langerhans is inhibited and thus the progression of the disease is slowed down [35].

Dendritic cells are one of the populations of antigen presenting cells in the body. They present a foreign antigen bound to the MHC type I or II molecule to T cells, activating them. In this connection, the effective inhibition of the dendritic cell function allows one to regulate the immune response. Tregs induce the formation of tolerogenic dendritic cells (cause the anergy of the T-effector interacting with the antigen-MHC complex, induce the formation of pTregs) or disrupt the process of antigen presentation by the dendritic cell [36].

Among the ways of metabolic disorders, one can single out the “stealing” of CD8+ lymphocytes by IL-2 (due to the higher density of the receptor for this interleukin on the Treg surface compared to CD8+ cells) [37].

Also, the mechanisms of metabolic disorders might include the induction of adenosine triphosphoric acid breakdown to adenosine by the enzymes on the Treg membrane. An increased concentration of adenosine reduces the proliferative activity of T lymphocytes, and also affects dendritic cells, suppressing their antigen-presenting ability [38].

Tregs can also disturb the supply of calcium ions to effector lymphocytes, disrupting their activation (since calcium-dependent transcription factors do not function) [39].

It is important to note that Tregs use these suppressive mechanisms in combination, and that these mechanisms continue to be actively studied.

The role of regulatory T cells in the development of graft-versus-host disease

Despite the improvement of donor selection algorithms (matching by human leukocyte antigen (HLA), and the preventive therapy, GVHD remains the leading cause of non-recurrent morbidity and mortality after alloHSCT [40].

There are 2 types of GVHD: aGVHD and chronic GVHD (cGVHD); aGVHD is characterized by the absence of signs characteristic of cGVHD. Acute GVHD form includes classic aGVHD (before day 100) and persistent, recurrent or late aGVHD (after day 100, often after the withdrawal of immunosuppression or the transfusion of donor lymphocytes). Chronic GVHD form includes classic cGVHD (no signs of aGVHD) and overlap syndrome (with the signs of both acute and cGVHD present) [41].

Pathogenetically, aGVHD is mediated by mature effector T cells of the donor, which are activated after encountering the recipient's alloantigens [42].

Pathogenetic mechanisms in cGVHD are not as well studied as in the acute one, primarily due to the fact that so far no animal model has reproduced all the features of the course of cGVHD in humans. However, the researchers were able to identify the main pathogenetic moments: the impairment of the tolerance mechanisms of both central (damage to the thymus by the conditioning, and/or in aGVHD, with the formation of T-lymphocytes directed against host antigens, which in turn leads to the activation of alloreactive B cells), and peripheral (Treg deficiency), as

well as the formation of fibrosis in target organs [43]. Next, we will review studies that have examined the role of Tregs in the GVHD pathogenesis.

It has been demonstrated in mouse models that the initial phase of cGVHD is associated with a significant decrease in Tregs in peripheral blood, and their number increases with time and against the impact of immunosuppressive therapy [44].

Another study with humans as the study subjects demonstrated that the Tregs to T helpers ratio is significantly lower at week 2 after alloHSCT in patients with subsequently developed aGVHD, compared to the patients in whom this complication was not observed later [45], in this connection a reduced Treg number can be considered as a predictor of the aGVHD development.

The study by A. C. Alho et al. [46] analyzed the CD3+ cell reconstitution in 107 patients after alloHSCT and demonstrated that the number of CD3+ cells recovered to normal within 2 years; however, it is important to note that in the T-cell compartment, CD8+ T cells recovered faster, and neither Tregs, nor T helpers reached normal levels during the 2-year follow-up period. The authors were also able to show that the Tregs to T-helpers ratio, and Tregs to CD8+ T cells ratio in patients with cGVHD were significantly lower than in patients without cGVHD.

Given that alloreactive lymphocytes implement an alloimmune response in tissues, it is interesting to study regulatory and effector populations both in blood, and also in target organs. So, in the study by K. Rieger et al., 95 duodenal and colon biopsies from 49 patients after alloHSCT were investigated [47]. In acute and cGVHD, an increased number of CD 8+ cells was noted compared to the samples obtained from patients without GVHD, which was comparable to the results of other authors [48], but this figure was also increased in diverticulitis and

cytomegalovirus colitis, which did not allow it to be used as a differential diagnostic marker. However, the researchers were able to find that in GVHD, the FoxP3+ to CD8+ ratio corresponded to that in samples obtained from patients without signs of inflammation in the duodenum and colon, while in diverticulitis and cytomegalovirus colitis there was an increase in this ratio following the expansion of CD8+ cells. The authors have interpreted the findings as follows: GVHD with intestinal involvement is associated with an insufficient Treg activation.

Other works are focused on the study of the graft cellular composition. Two similar studies conducted at approximately the same time by authors from USA [49] and from China [50] showed that the amount of Tregs in the graft affects the likelihood of developing aGVHD: the content of Tregs 10.0×10^6/kg and over reduces significantly the likelihood of developing aGVHD, and these patients also show a faster recovery of peripheral blood parameters after allo-HSCT.

Interestingly, mobilized with plerixaphor hematopoietic stem cell (HSC) transplants contain higher amounts of Tregs than when granulocyte colony stimulating factor (G-CSF) is used [51].

The role of regulatory T cells in graft acceptance

It is important to note that the regulatory potential of Tregs is so powerful that allogeneic HSCs can survive in unirradiated mice for about 30 days without additional immunosuppression, localizing next to the host's Tregs in bone marrow niches [52].

A number of studies have shown that Tregs promote the engraftment of HSCs. For example, in their work Y. Hirata et al. [53] studied the Treg population of the bone marrow niche in a mouse model. The authors demonstrated that a decrease in the number of Tregs of the bone marrow niche in the host body led to a significant decrease in donor

chimerism; and the degree of chimerism reduction was higher at 24 weeks after alloHSCT than at 9 weeks; and the transfusion of bone marrow niche Tregs increased the donor chimerism in peripheral blood. These observations confirm the ability of the bone marrow niche Tregs to provide a long-term immune protection of HSCs.

Another study in mouse models [54] showed that a Treg transfusion provides the engraftment of allogeneic HSCs, and also that temporary depletion of NK cells leads to the engraftment of HSCs without using a Treg transfusion. These observations led to the conclusion that Tregs facilitate the engraftment of HSCs, including by suppressing the function of NK cells. Interestingly, when the pool of NK cells had been restored after depletion, no allogeneic HSC rejection occurred, which was explained by the ability of Tregs to induce tolerance of host NK cells to the allograft – NK cells of HSC recipients who received Treg transfusion, and this changed the repertoire of receptors.

Thus, the Treg population is very important for the engraftment of allogeneic HSCs.

The role of regulatory T cells in implementing the graft-versus-tumor effect

It is known that the ability of Tregs to suppress the immune response contributes to carcinogenesis; for example, an increase in the number of circulating Tregs in patients suffering from cancer compared with healthy people has been reported [55]. Moreover, an increase in tumor-infiltrating Tregs is associated with poor prognosis and lower patient survival [56].

Worthwhile to note that an increase in the number of Tregs in the bone marrow and peripheral blood of patients with acute leukemia has been reported [57, 58]. However, Treg transfusions, as it have been

demonstrated in mouse models, while suppressing the capacity of alloreactive donor T cells to induce GVHD, do not abrogate their graft-versus-tumor (GVT) effector function [59]. Also, when using Treg transfusion in patients after alloHSCT, there was no decrease in GVT activity. [60–62]; these studies are described more detailed in the section on the clinical use of Tregs for the prevention of GVHD.

Effect of immunosuppressive agents on regulatory T cells

Considering the important role of Tregs in the pathogenesis of autoimmune and alloimmune complications, it is very important to understand the effect of various immunosuppressive agents on these cells.

Calcineurin inhibitors are the most commonly used immunosuppressants in transplantation of hematopoietic stem cells and solid organ transplantation. Studies have shown that calcineurin inhibitors reduce the number and functional activity of circulating Tregs [63], and with the discontinuation of these drugs, an increase in Tregs in the blood is noted [64]. This is also confirmed by the fact that the change of immunosuppressive therapy from calcineurin inhibitors to an antiproliferative agent, mycophenolate mofetil (MPM), is associated with an increase in the Treg pool [65], probably due to the induction of the conversion of T-helpers into Tregs [66]. However, there are studies demonstrating that MPM dose-dependently inhibits the proliferative activity and viability of Tregs [67].

Research by a group of German scientists [68] demonstrated in murine GVHD models that the Treg transfusion with using cyclosporine A increased a Treg accumulation at inflammation sites such as the lungs and liver, which may be useful in the treatment of GVHD involving these organs.

Drugs from the group of mammalian target of rapamycin (mTOR) inhibitors have a positive effect on Tregs. They promote the Treg expansion and support the Treg function in both ex vivo and in vivo studies [69], which makes GVHD prevention regimens based on mTOR inhibitors rather than calcineurin inhibitors promising.

Cyclophosphamide is gaining a strong position as a prophylactic agent against the GVHD development. The mechanism of cyclophosphamide action is based on the selective destruction of rapidly proliferating T cells, while Tregs "escape" this effect due to increased expression of the aldehyde dehydrogenase enzyme [70]. Interestingly, in a xenogenic transplantation model, the removal of Tregs from the graft abolished the protective effects of cyclosphosphamide [70].

Rabbit antithymocyte globulin (ATG) leads to depletion of circulating T lymphocytes, including Tregs, but the latter recover much faster than other populations, which is useful for preventing the development of GVHD [71]. However, in studies in vitro, when peripheral blood mononuclear cells were incubated with a low dose of rabbit ATG, an increase in the number of Tregs was noted (by converting CD4+CD25- T cells into CD4+CD25+ T cells), while when incubated with horse ATG, the number of Tregs decreased [72].

Glucocorticosteroids (GCS) today remain first-line drugs in the treatment of both acute and cGVHD, and, accordingly, their effect on Tregs is important. This effect was studied in patients receiving corticosteroids for autoimmune diseases. It was demonstrated that during therapy at a dose of 5 mg/day or more, there was an increase in the level of Tregs in peripheral blood in comparison with the level before the start of therapy, as well as in comparison with the group of patients who did not receive GCS [73].

On the contrary, when studying Tregs during therapy with methylprednisolone at a dose of 250 mg/day for 3 days (as a treatment for acute renal graft rejection), no quantitative change in the Treg population was recorded; however, some qualitative changes were described: an increase in the proportion of effector Tregs and a decrease in the proportion of central memory Tregs [74].

However, in the study of the German group mentioned above [68] in murine models of GVHD, the combination of methylprednisolone and Treg transfusion led to a decrease in Treg recruitment to inflammatory foci and a rapid deterioration in the condition of some animals.

Of interest is the effect produced on Tregs by hypomethylating drugs, which can be used as part of post-transplantation anti-relapse treatment. Azacitidine and decitabine induce FoxP3 expression in CD4+CD25- T cells both in vitro and in vivo [75]. Meanwhile, Tregs have been demonstrated in mouse models to be resistant to the antiproliferative effects of hypomethylating agents.

Clinical use of regulatory T cells as prevention of graft-versus-host disease

Given the undoubted role of Tregs in the pathogenesis of GVHD, the use of Tregs as a prevention and treatment of this complication has been very actively studied in recent years.

Studies in murine models have demonstrated the capacity of the Treg infusion to restrain the T-lymphocyte infusion-induced GVHD or alleviate its symptoms in a cell-dose and time-dependent manner [77].

An example of using of Tregs to prevent GVHD is a study called Orca-T [60]: on Day 0, patients underwent transfusion of selected CD34+ cells in combination with Tregs, the target dose of the latter being 3 $\times 10^6$/kg, and after 2 days they received the transfusion of CD3+ cells at a

dose of 3×10^6/kg. The study included 34 patients who underwent allo-HSCT from fully compatible donors (25 from a related fully compatible donor, 6 from an unrelated donor). Post-transplant immunosuppression included tacrolimus in 8 patients and sirolimus in 6. The comparison group (allo-HSCT in combination with methotrexate and tacrolimus) included 138 patients after allo-HSCT from a related (79 patients) and unrelated (59 patients) fully compatible donor. Faster graft healing was demonstrated in the Orca-T group, as well as a lower incidence of both grade 2-4 aGVHD (0% vs. 33%) and cGVHD (4% vs. 44%).

It is interesting that the use of Tregs is effective for the prevention of GVHD and in performing alloHSCT from haploidentical donors (haploHSCT), even without concomitant immunosuppressive therapy.

For example, in a study by M. Di Ianni et al. [61], 28 patients with hematological malignancies underwent alloHSCT from haploidentical donors according to the following scheme: selected Tregs on Day –4, and then selected CD34+ cells on Day 0 in combination with T-lymphocyte infusion. GVHD prophylaxis was not performed. Graft acceptance was noted in 26 of 28 patients; among those 26 patients, only 2 developed aGVHD and none developed cGVHD.

In the study of Italian researchers [62], 50 patients underwent haploHSCT for blood myeloid lineage tumour (MLT) according to the following scheme: Tregs at a dose of 2×10^6/kg on Day –4, CD3+ cells at a dose of 1×10^6/kg on Day –1, and selected CD34+ cells (median $10.7\pm3.4\times10^6$/kg) on day zero, without subsequent immunosuppressive therapy. Fifteen patients developed aGVHD grade 2 or higher; moderate/severe cGVHD developed in only 1 of 50 patients, and the two-year survival free from cGVHD and without the underlying disease progression was 75%.

It is important to note that all the studies cited in this part of the article [60-62] showed no negative effect of the Treg infusion on GVT activity, or on the likelihood of developing infectious complications.

Clinical use of regulatory T cells for the treatment of GVHD

The use of Tregs as a therapy for aGVHD has been well demonstrated in mouse models. For example, C. Riegel et al. in their study [78] showed that Tregs migrating to the lymphoid organs, as well as to the target organs of aGVHD (mainly of the gastrointestinal tract), suppress the proliferation of T cells and thereby reduce the clinical and histological signs of aGVHD, significantly improving survival.

There are no large studies on the use of Tregs in the treatment of human aGVHD, but there are reports of the effect of such therapy. For example, Polish authors reported that a Treg infusion to a patient suffering from grade 4 aGVHD allowed only a temporary improvement in the condition, but for the longest period among all used immunosuppressants [79].

Also, Treg transfusions are effective as a therapy for cGVHD. For example, in a study [80] there was shown the improvement and stabilization of the process when using Treg transfusion in patients suffering from cGVHD. In another study [81], patients with cGVHD received a transfusion of ex vivo expanded Tregs, and the process stabilization or clinical improvements were also reported.

To date, more than 40 studies on the role of Tregs in the prevention and treatment of both acute and cGVHD are available at clinicaltrials.gov.

Interestingly, the source of Tregs in a number of these studies was a "third" donor; the use of "third-party" Tregs is safe (does not increase

the risk of GVHD) and effective in controlling the development of GVHD [82].

Modification of regulatory T cells

The limiting factor in the use of Tregs is their small amount in the peripheral blood: the pool of Tregs in the peripheral blood of healthy people is only 5–10% of CD4+ T cells, and therefore it seems relevant to use Treg expansion both in vivo, and in vitro.

For Treg expansion in vivo, IL-2 can be used; this cytokine is extremely important for the development, proliferation and activity of Tregs. This approach has been shown to be effective in the treatment of cGVHD. So, for example, scientists from Korea, using low doses of IL-2 as a therapy for cGVHD, revealed an increase in the amount of Tregs in patient's peripheral blood, an increase in the Tregs to type 17 T helpers ratio, as well as a moderate clinical improvement [84]. Researchers from the USA demonstrated that an 8-week treatment with low doses of IL-2 caused an increase in Treg proliferation, as well as an increase in their resistance to apoptosis, meanwhile low doses of IL-2 had a minimal effect on T-conventional cells [85].

An interesting study by A.A. Kennedy-Nasser et al. [86] focused on the use of ultra-low doses of IL-2 (100,000–200,000 IU/m^2 3 times a week) from day 30 to 6–12 weeks after alloHSCT to prevent aGVHD. The study included 16 children who underwent alloHSCT from related (12 people) and unrelated (4 people) donors. The number of Tregs in peripheral blood during therapy increased an average from 4.8% to 11.1%, none of the treated patients developed grade 2–4 aGVHD compared with grade 4 in 33 (12 %) patients in the comparison group.

For the purpose of Treg expansion in vitro IL-2, rapamycin (mTOR inhibitor) have been used, as well as beads coated with antibodies against

CD3 and CD28 (the imitation of antigen-presenting cells (APC)) [87]. Tregs expanded in this way demonstrate clinical safety and efficacy.

It is also possible to induce the conversion of non-regulatory T cells into iTregs under laboratory conditions, however, this approach is limited by the instability (loss of FoxP3) and plasticity (acquisition of a T × 1/2/17-like phenotype and properties) of the obtained iTregs [13], and therefore studies are underway to stabilize FoxP3 [88].

The desire to obtain the maximum regulatory effect from Tregs has led to the creation and use of antigen-specific Tregs, which efficacy is higher than that of the polyclonal Tregs, as has been demonstrated in models of type I diabetes [89], autoimmune disease of the central nervous system [90], as well as in allogeneic transplantation models [91, 92].

For example, in a study [91] on a mouse model, the donor Treg expansion was performed in the presence of recipient's APCs, thereby facilitating the formation of Tregs specific to the recipient's antigens, capable of controlling GVHD. On the contrary, in another study [92], alloantigen-specific Tregs contributed to inhibiting the skin graft rejection.

However, the expansion of antigen-specific Tregs is limited due to a low number of precursors. The authors found three ways to solve this problem [93]: two of them are based on the redirection of polyclonal Tregs by means of forming an artificial receptor: a chimeric antigen receptor (CAR) or engineered TCR, and the third pathway is based on the conversion of antigen-specific T lymphocytes into Tregs by the induction of FOXP 3.

So far, only CAR-Tregs have found an application in the field of alloHSCT. In the study, K.G. MacDonald and al. [94] demonstrated in a xenogenic transplantation model that Tregs constructed by the CAR targeted the HLA-A2 (usually mismatched in alloHSCT) induce

alloantigen-specific suppression, preventing GVHD. However, there is no doubt that two other methods for the expansion of antigen-specific Tregs will also take their rightful place in the prevention and treatment of alloHSCT complications.

Conclusions

1. Regulatory T cells are a unique population of lymphocytes that provide peripheral tolerance. Probably, there are still many discoveries in store for us in the field of mechanisms by which regulatory T cells realize their functions. However, to date, the therapies using regulatory T cells or targeted them have already taken a significant position in the practice of physicians around the world.

2. Regulatory T cells are a promising method for the prevention and treatment of graft versus host disease, which allows one to control this severe alloimmune complication without losing the graft versus tumor effect. This method is limited by the financial component, as well as by the low content of the population of regulatory T cells in the peripheral blood, which requires the use of cell expansion or their modification. Further studies in this area may improve the outcomes of allogeneic hematopoietic stem cell transplantation.

References

1. Vignali DAA, Collison LW, Workman CJ. How regulatory T cells work. *Nat Rev Immunol.* 2008;8(7):523. PMID: 18566595 https://doi.org/10.1038/NRI2343

2. Gershon RK, Kondo K. Infectious immunological tolerance. *Immunology.* 1971;21(6):903–914. PMID: 4943147

3. Nishizuka Y. A novel experimental system of organ-localized autoimmune diseases in the mouse. *Acta Pathol Jpn.* 1982;32 Suppl 1:211–222. PMID: 6764999

4. Powrie F, Leach MW, Mauze S, Caddie LB, Coffman RL. Phenotypically distinct subsets of CD4+ T cells induce or protect from chronic intestinal inflammation in C. B-17 scid mice. *Int Immunol.* 1993;5(11):1461–1471. PMID: 7903159 https://doi.org/10.1093/intimm/5.11.1461

5. Almeida ARM, Legrand N, Papiernik M, Freitas AA. Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. *J Immunol.* 2002;169(9):4850–4860. PMID: 12391195 https://doi.org/10.4049/jimmunol.169.9.4850

6. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. *Science.* 2003;299(5609):1057–1061. PMID: 12522256 https://doi.org/10.1126/science.1079490

7. Liu W, Putnam AL, Xu-yu Z, Szot GL, Lee MR, Zhu S, et al. CD127 expression inversely correlates with FoxP3 and suppressive function of human CD4+ T reg cells. *J Exp Med.* 2006;203(7):1701–1711. PMID: 16818678 https://doi.org/10.1084/jem.20060772

8. Kalampokis I, Yoshizaki A, Tedder TF. IL-10-producing regulatory B cells (B10 cells) in autoimmune disease. *Arthritis Res Ther.* 2013;15Suppl1(Suppl 1):S1. PMID: 23566714 ttps://doi.org/10.1186/ar3907

9. Mishra S, Srinivasan S, Ma C, Zhang N. CD8+ regulatory T cell – a mystery to be revealed. *Front Immunol.* 2021;12: 708874. PMID: 34484208 https://doi.org/10.3389/FIMMU.2021.708874

10. Takahashi T, Sakaguchi S. Naturally arising CD25+CD4+ regulatory T cells in maintaining immunologic self-tolerance and preventing autoimmune disease. *Curr Mol Med.* 2003;3(8):693–706. PMID: 14682491 https://doi.org/10.2174/1566524033479429

11. Sakaguchi S, Sakaguchi N, Shimizu J, Yamazaki S, Sakihama T, Itoh M, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. *Immunol Rev.* 2001;182:18–32. PMID: 11722621 https://doi.org/10.1034/J.1600-065X.2001.1820102.X

12. Saleh R, Elkord E. FoxP3+ T regulatory cells in cancer: prognostic biomarkers and therapeutic targets. *Cancer Lett.* 2020;490:174–85. PMID: 32721551 https://doi.org/10.1016/J.canlet.2020.07.022

13. Dominguez-Villar M, Hafler DA. Regu-latory T cells in autoimmune disease. *Nat Immunol.* 2018;19(7):665–673. PMID: 29925983 https://doi.org/10.1038/S41590-018-0120-4

14. Schwarz BA, Bhandoola A. Trafficking from the bone marrow to the thymus: a prerequisite for thymopoiesis. *Immunol Rev.* 2006;209(1):47–57. PMID: 16448533 https://doi.org/10.1111/J.0105-2896.2006.00350.X

15. Baldwin TA, Hogquist KA, Jameson SC. The fourth way? Harnessing aggressive tendencies in the thymus. *J Immunol.* 2004;173(11):6515–6520. PMID: 15557139 https://doi.org/10.4049/JIMMUNOL.173.11.6515

16. Lio CWJ, Hsieh CS. A two-step process for thymic regulatory T cell development. *Immunity.* 2008;28(1):100–111. PMID: 18199417 https://doi.org/10.1016/j.immuni.2007.11.021

17. Fazilleau N, Bachelez H, Gougeon ML, Viguier M. Cutting edge: size and diversity of CD4+CD25high Foxp3+ regulatory T cell

repertoire in humans: evidence for similarities and partial overlapping with CD4+CD25- T cells. *J Immunol.* 2007;179(6):3412–3416. PMID: 17785774 https://doi.org/10.4049/jimmunol.179.6.3412

18. Caramalho Í, Nunes-Cabaço H, Foxall RB, Sousa AE. Regulatory T-cell development in the human thymus. *Front Immunol.* 2015;6:395. PMID: 26284077 https://doi.org/10.3389/fimmu.2015.00395

19. Chen WJ, Jin W, Hardegen N, Lei KJ, Li L, Marinos N, et al. Conversion of peripheral CD4+CD25- naive T cells to CD4+CD25+ regulatory T cells by TGF-beta induction of transcription factor Foxp3. *J Exp Med.* 2003;198(12):1875–1886. PMID: 14676299 https://doi.org/10.1084/jem.20030152

20. Samstein RM, Josefowicz SZ, Arvey A, Treuting PM, Rudensky AY. Extrathymic generation of regulatory T cells in placental mammals mitigates maternal-fetal conflict. *Cell.* 2012;150(1):29–38. PMID: 22770213 https://doi.org/10.1016/J.CELL.2012.05.031

21. Cebula A, Seweryn M, Rempala GA, Pabla SS, McIndoe RA, Denning TL, et al. Thymus-derived regulatory T cells contribute to tolerance to commensal microbiota. *Nature.* 2013;497(7448):258–262. PMID: 23624374 https://doi.org/10.1038/NATURE12079

22. Zegarra-Ruiz DF, Kim DV, Norwood K., Kim M, Wu WJH, Saldana-Morales FB, et al. Thymic development of gut-microbiota-specific T cells. *Nature.* 2021;594(7863):413–417. PMID: 33981034 https://doi.org/10.1038/S41586-021-03531-1

23. Komatsu N, Okamoto K, Sawa S, Nakashima T, Oh-hora M, Kodama T, et al. Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. *Nat Med.* 2014;20(1):62–68. PMID: 24362934 https://doi.org/10.1038/NM.3432

24. Wyss L, Stadinski BD, King CG, Schallenberg S, Mccarthy NI, Lee JY, et al. Affinity for self antigen selects Treg cells with distinct

functional properties. *Nat Immunol.* 2016;17(9):1093–1101. PMID: 27478940 https://doi.org/10.1038/NI.3522

25. Thome JJC, Yudanin N, Ohmura Y, Kubota M, Grinshpun B, Sathaliyawala T, et al. Spatial map of human T cell compartmentalization and maintenance over decades of life. *Cell.* 2014;159(4):814–828. PMID: 25417158 https://doi.org/10.1016/j.cell.2014.10.026

26. Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, et al. Functional delineation and differentiation dynamics of human CD4+ T cells expressing the FoxP3 transcription factor. *Immunity.* 2009;30(6):899–911. PMID: 19464196 https://doi.org/10.1016/j.immuni.2009.03.019

27. Seddiki N, Santner-Nanan B, Tangye SG, Alexander SI, Solomon M, Lee S, et al. Persistence of naive CD45RA+ regulatory T cells in adult life. *Blood.* 2006;107(7):2830–2838. PMID: 16332974 https://doi.org/10.1182/BLOOD-2005-06-2403

28. Zhao DM, Thornton AM, DiPaolo RJ, Shevach EM. Activated CD4+CD25+ T cells selectively kill B lymphocytes. *Blood.* 2006;107(10):3925–3932. PMID: 16418326 https://doi.org/10.1182/blood-2005-11-4502

29. Cao X, Cai SF, Fehniger TA, Song J, Collins LI, Piwnica-Worms DR, et al. Granzyme B and perforin are important for regulatory T cell-mediated suppression of tumor clearance. *Immunity.* 2007;27(4):635–646. PMID: 17919943 https://doi.org/10.1016/j.immuni.2007.08.014

30. Drokov MY, Parovichnikova EN, Kuzmina LA, Galtseva IV, Vasilieva VA, Mikhaltsova ED, et al. Role of granzyme B in T regulatory cells in patients after allogeneic stem cell transplantation. *Gematologiya I Transfusiologiya.* 2016;61(1):32–37. (In Russ.). https://doi.org/10.18821/0234-5730-2016-61-1-32-37

31. Zhu Z, Zhang Y, Ye J, Wang X, Fu X, Yin Y, et al. IL-35 promoted STAT3 phosphorylation and IL-10 production in B cells, but its production was reduced in patients with coronary artery diseases. *Hum Immunol.* 2018;79(12):869–875. PMID: 30316971 https://doi.org/10.1016/j.humimm.2018.10.009

32. Asseman C, Mauze S, Leach MW, Coffman RL, Powrie F. An essential role for interleukin 10 in the function of regulatory T cells that inhibit intestinal inflammation. *J Exp Med.* 1999;190(7):995–1003. PMID: 10510089 https://doi.org/10.1084/JEM.190.7.995

33. Hawrylowicz CM, O'Garra A. Potential role of interleukin-10-secreting regulatory T cells in allergy and asthma. *Nat Rev Immunol.* 2005;5(4):271–283. PMID: 15775993 https://doi.org/10.1038/NRI1589

34. Loser K, Apelt J, Voskort M, Mohaupt M, Balkow S, Schwarz T, et al. IL-10 controls ultraviolet-induced carcinogenesis in mice. *J Immunol.* 2007;179(1):365–371. PMID: 17579057 https://doi.org/10.4049/jimmunol.179.1.365

35. Green EA, Gorelik L, McGregor CM, Tran EH, Flavell RA. CD4+CD25+ T regu-latory cells control anti-islet CD8+ T cells through TGF-beta-TGF-beta receptor interactions in type 1 diabetes. *Proc Natl Acad Sci USA.* 2003;100(19):10878–10883. PMID: 12949259 https://doi.org/10.1073/PNAS.1834400100

36. Akkaya B, Oya Y, Akkaya M, Al Souz J, Holstein AH, Kamenyeva O, et al. Regulatory T cells mediate specific suppression by depleting peptide-MHC class II from dendritic cells. *Nat Immunol.* 2019;20(2):218–231. PMID: 30643268 https://doi.org/10.1038/S41590-018-0280-2

37. Chinen T, Kannan AK, Levine AG, Fan X, Klein U, Zheng Y, et al. An essential role for the IL-2 receptor in T reg cell function. *Nat*

Immunol. 2016;17(11):1322–1333. PMID: 27595233 https://doi.org/10.1038/NI.3540

38. Ernst PB, Garrison JC, Thompson LF. Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology. *J Immunol.* 2010;185(4):1993–1998. PMID: 20686167 https://doi.org/10.4049/jimmunol.1000108

39. Schwarz A, Schumacher M, Pfaff D, Schumacher K, Jarius S, Balint B, et al. Fine-tuning of regulatory T cell function: the role of calcium signals and naive regulatory T cells for regulatory T cell deficiency in multiple sclerosis. *J Immunol.* 2013;190(10):4965–4970. PMID: 23576680 https://doi.org/10.4049/JIMMUNOL.1203224

40. Ferrara JL, Levine JE, Reddy P, Holler E. Graft-versus-host disease. *Lancet.* 2009;373(9674):1550–1561. PMID: 19282026 https://doi.org/10.1016/S0140-6736(09)60237-3

41. Jagasia MH, Greinix HT, Arora M, Williams KM, Wolff D, Cowen EW, et al. National institutes of health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. The 2014 diagnosis and staging working group report. *Biol Blood Marrow Transplant.* 2015;21(3):389–401.e1. PMID: 25529383 https://doi.org/10.1016/J.BBMT.2014.12.001

42. Zeiser R, Blazar BR. Acute graft-versus-host disease – biologic process, prevention, and therapy. *N Engl J Med.* 2017;377(22):2167–2179. PMID: 29171820 https://doi.org/10.1056/NEJMRA1609337

43. Cooke KR, Luznik L, Sarantopoulos S, Hakim FT, Jagasia M, Fowler DH, et al. The Biology of chronic graft-versus-host disease: a task force report from the National institutes of health consensus development project on criteria for clinical trials in chronic graft-versus-host disease. *Biol Blood Marrow Transplant.* 2017;23(2):211–234. PMID: 27713092 https://doi.org/10.1016/J.BBMT.2016.09.023

44. Schneider M, Munder M, Karakhanova S, Ho AD, Goerner M. The initial phase of graft-versus-host disease is associated with a decrease of CD4+CD25+ regulatory T cells in the peripheral blood of patients after allogeneic stem cell transplantation. *Clin Lab Haematol.* 2006;28(6):382–390. PMID: 17105491 https://doi.org/10.1111/J.1365-2257.2006.00825.X

45. Fujioka T, Tamaki H, Ikegame K, Yoshihara S, Taniguchi K, Kaida K, et al. Frequency of CD4(+)FOXP3(+) regulatory T-cells at early stages after HLA-mismatched allogeneic hematopoietic SCT predicts the incidence of acute GVHD. *Bone Marrow Transplant.* 2013;48(6):859–864. PMID: 23165499 https://doi.org/10.1038/BMT.2012.232

46. Alho AC, Kim HT, Chammas MJ, Reynolds CG, Matos TR, Forcade E, et al. Unbalanced recovery of regulatory and effector T cells after allogeneic stem cell transplantation contributes to chronic GVHD. *Blood.* 2016;127(5):646–657. PMID: 26670634 https://doi.org/10.1182/BLOOD-2015-10-672345

47. Rieger K, Loddenkemper C, Maul J, Fietz T, Wolff D, Terpe H, et al. Mucosal FOXP3+ regulatory T cells are numerically deficient in acute and chronic GVHD. *Blood.* 2006;107(4):1717–1723. PMID: 16278306 https://doi.org/10.1182/BLOOD-2005-06-2529

48. Forbes GM, Erber WN, Herrmann RP, Davies JM, Collins BJ. Immunohistochemical changes in sigmoid colon after allogeneic and autologous bone marrow transplantation. *J Clin Pathol.* 1995;48(4):308–313. PMID: 7615847 https://doi.org/10.1136/JCP.48.4.308

49. Rezvani K, Mielke S, Ahmadzadeh M, Kilical Y, Savani BN, Zeilah J. High donor FOXP3-positive regulatory T-cell (Treg) content is associated with a low risk of GVHD following HLA-matched allogeneic SCT. *Blood.* 2006;108(4):1291–1297. PMID: 16627754 https://doi.org/10.1182/BLOOD-2006-02-003996

50. Yang K, Fan Z-P, Liu Q-F, Zhang Y. Effect of donor CD4+CD25+ regulatory T cells on hematopoietic and immune reconstitution, GVHD and disease-free survival after allogeneic hematopoietic stem cell transplantation. *Nan Fang Yi Ke Da Xue Xue Bao*. 2008;28(4):537–541. PMID: 18495584

51. Le Texier L, Lineburg KE, Cao B, McDonald-Hyman C, Mouttie LLEl, Nicholls J, et al. Autophagy-dependent regulatory T cells are critical for the control of graft-versus-host disease. *JCI Insight.* 2016;1(15). PMID: 27699243 https://doi.org/10.1172/JCI.INSIGHT.86850

52. Fujisaki J, Wu J, Carlson AL, Silberstein L, Putheti P, Larocca R, et al. In vivo imaging of Treg cells providing immune privilege to the haematopoietic stem-cell niche. *Nature.* 2011;474(7350):216–219. PMID: 21654805 https://doi.org/10.1038/NATURE10160

53. Hirata Y, Furuhashi K, Ishii H, Li HW, Pinho S, Ding L, et al. CD150high bone marrow Tregs maintain hematopoietic stem cell quiescence and immune privilege via adenosine. *Cell Stem Cell.* 2018;22(3):445–453.e5. PMID: 29456159 https://doi.org/10.1016/J.STEM.2018.01.017

54. Mahr B, Pilat N, Maschke S, Granofszky N, Schwarz C, Unger L, et al. Regulatory T cells promote natural killer cell education in mixed Chimeras. *Am J Transplant.* 2017;17(12):3049–3059. PMID: 28489338 https://doi.org/10.1111/AJT.14342

55. Kotsakis A, Koinis F, Katsarou A, Gioulbasani M, Aggouraki D, Kentepozidis N, et al. Prognostic value of circula-ting regulatory T cell subsets in untrea-ted non-small cell lung cancer patients. *Sci Rep.* 2016;6:39247. PMID: 27976733 https://doi.org/10.1038/SREP39247

56. Nair VS, Elkord E. Immune checkpoint inhibitors in cancer therapy: a focus on T-regulatory cells. *Immunol Cell Biol.* 2018;96(1):21–33. PMID: 29359507 https://doi.org/10.1111/IMCB.1003

57. Idris SZ, Hassan N, Lee LJ, Md Noor S, Osman R, Abdul-Jalil M, et al. Increased regulatory T cells in acute lymphoblastic leukaemia patients. *Hematology.* 2016;21(4):206–212. PMID: 26907959 https://doi.org/10.1080/10245332.2015.1101965

58. Wan Y, Zhang C, Xu Y, Wang M, Rao Q, Xing H, et al. Hyperfunction of CD4 CD25 regulatory T cells in de novo acute myeloid leukemia. *BMC Cancer*. 2020;20(1):472. PMID: 32456622 https://doi.org/10.1186/S12885-020-06961-8

59. Edinger M, Hoffmann P, Ermann J, Drago K, Garrison Fathman C, Strober S, et al. CD4+CD25+ regulatory T cells preserve graft-versus-tumor activity while inhibiting graft-versus-host disease after bone marrow transplantation. *Nat Med.* 2003;9(9):1144–1450. PMID: 12925844 https://doi.org/10.1038/NM915

60. Meyer EH, Hoeg R, Moroz A, Xie BJ, Wu HH, Pawar R, et al. 88 – Orca-T, a precision Treg-engineered donor product, in myeloablative HLA-matched transplantation prevents acute GVHD with less immunosuppression in an early multicenter experience. *Transplant Cell Ther.* 2021;27(3Suppl):S84–S85. https://doi.org/10.1016/S2666-6367(21)00114-7

61. Di Ianni M, Falzetti F, Carotti A, Terenzi A, Castellino F, Bonifacio E, et al. Tregs prevent GVHD and promote immune reconstitution in HLA-haploidentical transplantation. *Blood.* 2011;117(14):3921–3928. PMID: 21292771 https://doi.org/10.1182/BLOOD-2010-10-311894

62. Pierini A, Ruggeri L, Carotti A, Falzetti F, Saldi S, Terenzi A, et al. Haploidentical age-adapted myeloablative transplant and regulatory

and effector T cells for acute myeloid leukemia. *Blood Adv*. 2021;5(5):1199–2208. PMID: 33646302 https://doi.org/10.1182/bloodadvances.2020003739

63. Segundo DS, Ruiz JC, Izquierdo M, Fernández-Fresnedo G, Gómez-Alamillo C, Merino R, et al. Calcineurin inhibitors, but not rapamycin, reduce percentages of CD4+CD25+FOXP3+ regulatory T cells in renal transplant recipients. *Transplantation.* 2006;82(4):550–557. PMID: 16926600 https://doi.org/10.1097/01.TP.0000229473.95202.50

64. Pascual J, Bloom D, Torrealba J, Brahmbhatt R, Chang Z, Sollinger HW, et al. Calcineurin inhibitor withdrawal after renal transplantation with alemtuzumab: clinical outcomes and effect on T-regulatory cells. *Am J Transplant.* 2008;8(7):1529–1536. PMID: 18510645 https://doi.org/10.1111/J.1600-6143.2008.02260.X

65. Demirkiran A, Sewgobind VDKD, Van Der Weijde J, Kok A, Baan CC, Kwekkeboom J, et al. Conversion from calcineurin inhibitor to mycophenolate mofetil-based immunosuppression changes the frequency and phenotype of CD4+FOXP3+ regulatory T cells. *Transplantation.* 2009;87(7):1062–1068. PMID: 19352129 https://doi.org/10.1097/TP.0B013E31819D2032

66. He X, Smeets RL, Koenen HJPM, Vink PM, Wagenaars J, Boots AMH, et al. Mycophenolic acid-mediated suppression of human CD4+ T cells: more than mere guanine nucleotide deprivation. *Am J Transplant.* 2011;11(3):439–449. PMID: 21342445 https://doi.org/10.1111/J.1600-6143.2010.03413.X

67. Scottà C, Fanelli G, Hoong SJ, Romano M, Lamperti EN, Sukthankar M, et al. Impact of immunosuppressive drugs on the therapeutic efficacy of ex vivo expanded human regulatory T cells. *Haematologica.* 2016;101(1):91–100. PMID: 26471483 https://doi.org/10.3324/haematol.2015.128934

68. Landwehr-Kenzel S, Zobel A, Schmitt-Knosalla I, Forke A, Hoffmann H, Schmueck-Henneresse M, et al. Cyclosporine A but not corticosteroids support efficacy of ex vivo expanded, adoptively transferred human Tregs in GVHD. *Front Immunol.* 2021;12:716629. PMID: 34707604 https://doi.org/10.3389/FIMMU.2021.716629

69. Zhang P, Tey SK, Koyama M, Kuns RD, Olver SD, Lineburg KE, et al. Induced regulatory T cells promote tolerance when stabilized by rapamycin and IL-2 in vivo. *J Immunol.* 2013;191(10):5291–5303. PMID: 24123683 https://doi.org/10.4049/jimmunol.1301181

70. Kanakry CG, Ganguly S, Zahurak M, Bolaños-Meade J, Thoburn C, Perkins B, et al. Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide. *Sci Transl Med.* 2013;5(211):211ra157. PMID: 24225944 https://doi.org/10.1126/scitranslmed.3006960

71. Gurkan S, Luan Y, Dhillon N, Allam SR, Montague T, Bromberg JS, et al. Immune reconstitution following rabbit antithymocyte globulin. *Am J Transplant.* 2010;10(9):2132–2141. PMID: 20883548 https://doi.org/10.1111/J.1600-6143.2010.03210.X

72. Feng X, Kajigaya S, Solomou EE, Keyvanfar K, Xu X, Raghavachari N, et al. Rabbit ATG but not horse ATG promotes expansion of functional CD4+CD25highFOXP3+ regulatory T cells in vitro. *Blood.* 2008;111(7):3675–3683. PMID: 18250226 https://doi.org/10.1182/BLOOD-2008-01-130146

73. Suárez A, López P, Gómez J, Gutiérrez C. Enrichment of CD4+ CD25high T cell population in patients with systemic lupus erythematosus treated with glucocorticoids. *Ann Rheum Dis.* 2006;65(11):1512–1517. PMID: 16606650 https://doi.org/10.1136/ARD.2005.049924

74. Seissler N, Schmitt E, Hug F, Sommerer C, Zeier M, Schaier M, et al. Methylprednisolone treatment increases the proportion of the highly suppressive HLA-DR(+)-Treg-cells in transplanted patients. *Transpl Immunol.* 2012;27(4):157–161. PMID: 23022208 https://doi.org/10.1016/J.TRIM.2012.09.003

75. Choi J, Ritchey J, Prior JL, Holt M, Shannon WD, Deych E, et al. In vivo administration of hypomethylating agents mitigate graft-versus-host disease without sacrificing graft-versus-leukemia. *Blood.* 2010;116(1):129–139. PMID: 20424188 https://doi.org/10.1182/BLOOD-2009-12-257253

76. Cooper ML, Choi J, Karpova D, Vij K, Ritchey J, Schroeder MA, et al. Azacitidine mitigates graft-versus-host disease via differential effects on the proliferation of T effectors and natural regulatory T cells in vivo. *J Immunol.* 2017;198(9):3746–3754. PMID: 28330901 https://doi.org/10.4049/JIMMUNOL.1502399

77. Nguyen VH, Zeiser R, DaSilva DL, Chang DS, Beilhack A, Contag CH, et al. In vivo dynamics of regulatory T-cell trafficking and survival predict effective strategies to control graft-versus-host disease following allogeneic transplantation. *Blood.* 2007;109(6):2649–2656. PMID: 17095616 https://doi.org/10.1182/BLOOD-2006-08-044529

78. Riegel C, Boeld TJ, Doser K, Huber E, Hoffmann P, Edinger M. Efficient treatment of murine acute GVHD by in vitro expanded donor regulatory T cells. *Leukemia.* 2020;34(3):895–908. PMID: 31719679 https://doi.org/10.1038/S41375-019-0625-3

79. Trzonkowski P, Bieniaszewska M, Juścińska J, Dobyszuk A, Krzysty-niak A, Marek N, et al. First-in-man clinical results of the treatment of patients with graft versus host di-sease with human ex vivo expanded CD4+CD25+CD127- T regulatory cells. *Clin Immunol.* 2009;133(1):22–26. PMID: 19559653 https://doi.org/10.1016/J.CLIM.2009.06.001

80. Marinelli Busilacchi E, Costantini A, Viola N, Costantini B, Olivieri J, Butini L, et al. Immunomodulatory effects of tyrosine kinase inhibitor in vitro and in vivo study. *Biol Blood Marrow Transplant.* 2018;24(2):267–275. PMID: 29128554 https://doi.org/10.1016/J.BBMT.2017.10.039

81. Theil A, Tuve S, Oelschlägel U, Maiwald A, Döhler D, Oßmann D, et al. Adoptive transfer of allogeneic regulatory T cells into patients with chronic graft-versus-host disease. *Cytotherapy.* 2015;17(4):473–486. PMID: 25573333 https://doi.org/10.1016/J.JCYT.2014.11.005

82. Lim JY, Im KIl, Song Y, Kim N, Nam YS, Jeon YW, et al. Third-party regulatory T cells prevent murine acute graft-versus-host disease. *Korean J Intern Med.* 2018;33(5):980–989. PMID: 29050459 https://doi.org/10.3904/KJIM.2016.319

83. Ng WF, Duggan PJ, Ponchel F, Matarese G, Lombardi G, David EA, et al. Human CD4+CD25+ cells: a naturally occurring population of regulatory T cells. *Blood.* 2001;98(9):2736–2744. PMID: 11675346 https://doi.org/10.1182/BLOOD.V98.9.2736

84. Nelson BH. IL-2, regulatory T cells, and tolerance. *J Immunol.* 2004;172(7):3983–3988. PMID: 15034008 https://doi.org/10.4049/JIMMUNOL.172.7.3983

85. Matsuoka KI, Koreth J, Kim HT, Bascug G, McDonough S, Kawano Y, et al. Low-dose interleukin-2 therapy restores regulatory T cell homeostasis in patients with chronic graft-versus-host disease. *Sci Transl Med.* 2013;5(179):179ra43. PMID: 23552371 https://doi.org/10.1126/SCITRANSLMED.3005265

86. Kennedy-Nasser AA, Ku S, Castillo-Caro P, Hazrat Y, Wu MF, Liu H, et al. Ultra low-dose IL-2 for GVHD prophylaxis after allogeneic hematopoietic stem cell transplantation mediates expansion of regulatory

T cells without diminishing antiviral and antileukemic activity. *Clin Cancer Res.* 2014;20(8):2215–2225. PMID: 24573552 https://doi.org/10.1158/1078-0432.CCR-13-3205

87. Morales JM M, Münch N, Peter K, Freund D, Oelschlägel U, Hölig K, et al. Automated clinical grade expansion of regulatory T cells in a fully closed system. *Front Immunol.* 2019;10:38. PMID: 30778344 https://doi.org/10.3389/FIMMU.2019.00038

88. Someya K, Nakatsukasa H, Ito M, Kondo T, Tateda K, Akanuma T, et al. Improvement of Foxp3 stability through CNS2 demethylation by TET enzyme induction and activation. *Int Immunol.* 2017;29(8):365–375. PMID: 29048538 https://doi.org/10.1093/INTIMM/DXX049

89. Tang Q, Henriksen KJ, Bi M, Finger EB, Szot G, Ye J, et al. In vitro-expanded antigen-specific regulatory T cells suppress autoimmune diabetes. *J Exp Med.* 2004;199(11):1455–1465. PMID: 15184499 https://doi.org/10.1084/JEM.20040139

90. Stephens LA, Malpass KH, Anderton SM. Curing CNS autoimmune disease with myelin-reactive Foxp3+ Treg. *Eur J Immunol.* 2009;39(4):1108–1117. PMID: 19350586 https://doi.org/10.1002/EJI.200839073

91. Trenado A, Charlotte F, Fisson S, Yagello M, Klatzmann D, Salomon BL, et al. Recipient-type specific CD4+CD25+ regulatory T cells favor immune reconstitution and control graft-versus-host disease while maintaining graft-versus-leukemia. *J Clin Invest.* 2003;112(11):1688–1696. PMID: 14660744 https://doi.org/10.1172/JCI17702

92. Sagoo P, Ali N, Garg G, Nestle FO, Lechler RI, Lombardi G. Human regulatory T cells with alloantigen specificity are more potent inhibitors of alloimmune skin graft damage than polyclonal regulatory T cells. *Sci Transl Med.* 2011;3(83):83ra42. PMID: 21593402 https://doi.org/10.1126/SCITRANSLMED.3002076

93. Raffin C, Vo LT, Bluestone JA. Treg cell-based therapies: challenges and perspectives. *Nat Rev Immunol.* 2020;20(3):158–172. PMID: 31811270 https://doi.org/10.1038/S41577-019-0232-6

94. MacDonald KG, Hoeppli RE, Huang Q, Gillies J, Luciani DS, Orban PC, et al. Alloantigen-specific regulatory T cells generated with a chimeric antigen receptor. *J Clin Invest.* 2016;126(4):1413–1424. PMID: 26999600 https://doi.org/10.1172/JCI82771

Information about the authors

Olga S. Karavaeva, Postgraduate, Hematologist, National Medical Research Center for Hematology, https://orcid.org/0000-0001-8158-8210, olga.starikova.1994@mail.ru

40%, writing the text of the manuscript, review of publications on the topic of the article

Mikhail Yu. Drokov, Cand. Sci. (Med.), Hematologist, Head of the Sector for Scientific Research in Chemoblastosis Chemotherapy, Hematopoietic Depressions, and Bone Marrow Transplantation, National Medical Research Center for Hematology, https://orcid.org/0000-0001-9431-8316

30%, development of the review concept and design, editing the manuscript

Ekaterina G. Khamaganova, Dr. Sci. (Biol.), Immunogeneticist, Head of the Tissue Typing Laboratory, National Medical Research Center for Hematology, https://orcid.org/0000-0002-0110-3314

30%, development of the review concept and design, editing the manuscript

The article was received on July 2, 2022;
approved after reviewing July 27, 2022;
accepted for publication September 28, 2022