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Regulatory T cells in allogeneic hematopoietic stem cell transplantation: From the lab to the clinic

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ABSTRACT

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is a curable strategy for the treatment of hematological malignancies and nonmalignant diseases. However, graft-versus-host disease (GVHD) and relapse are still two major causes of morbidity and mortality after allo-HSCT, and both restrict the improvement of transplant outcomes. Regulatory T cells (Tregs) has been successfully used in allo-SCT settings. In this review, we summarize recent advances in experimental studies that have evaluated the roles played by Tregs in the establishment of novel transplant modalities, the prevention of GVHD and the enhancement of immune reconstitution. We also discuss the application of Tregs in clinical to prevent acute GVHD, treat chronic GVHD, as well as enhance immune reconstitution and decrease leukemia relapse, all of which lead to improving transplant outcomes.

1. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) remains a curable therapy for the majority of hematological malignancies and nonmalignant diseases [1–4]. The establishment of haploidentical modalities has ensured that nearly everyone has a transplant donor [5–7]. However, transplant complications, including graft-versus-host disease (GVHD) and leukemia relapse, are the main causes of morbidity and mortality after allo-HSCT [8,9]. The key issues underlying these complications are immune tolerance and immune reconstitution [10,11]. A number of studies support the notion that inducing immune tolerance and enhancing immune recovery may not only contribute to the establishment of novel haploidentical transplant protocols but may also lead to strategies to deal with transplant complications, such as GVHD and leukemia relapse [12,13].

Regulatory T cells (Tregs), including natural and inducible Tregs, are the main types of regulatory cells [14]. Tregs were first described by Sakaguchi et al. [15] in 1995. Over the past two decades, Tregs have been confirmed to be related to immune tolerance in the settings of not only autoimmune diseases [10,16–18] and solid organ transplantation [19–21] but also for the allo-HSCT modality [22–40]. In this review, we focus on the role of Tregs in allo-HSCT [22–40], such as establishing novel transplant modalities [38–40] preventing GVHD and enhancing immune reconstitution, which contribute to improve transplant outcomes (Table 1).

2. Mechanisms of Tregs mediated T cell suppression

Tregs, which are characterized by high and stable expression of the interleukin (IL)-2 receptor α chain (CD25) and the master transcription factor forkhead box protein 3 (FoxP3), account for 5–10% of circulating $CD4^+$ cells and are crucial for the maintenance of central and peripheral immune tolerance [41]. The mechanisms by which Tregs suppress T cells include the following [42,43]: i) inhibiting T cell activation via secretion of anti-inflammatory cytokines, such as interleukin-10 (IL-10), IL-35 and transforming growth factor-β (TGF-β); ii) preventing cytokine-induced production/proliferation of T cells through release of extracellular vesicles from endosomal membranes; iii) modulating antigen-presenting cell (APC), especially dendritic cells (DC), maturation and function via interaction of cytotoxic T lymphocyte antigen (CTLA)-4 on Tregs with its ligand CD80/86 on APCs; the APCs can deliver a negative signal for T cell activation; iv) inducing T cell apoptosis via granzyme A/B and perforin, the Fas/Fas-ligand pathway, the galectin-9/T cell immunoglobulin and mucin domain-3 (TIM-3) pathway and the inducible cyclic adenosine monophosphate (cAMP) early repressor (TRAIL), as well as through high affinity CD25-dependent cytokine deprivation-mediated pathway; and v) disrupting metabolic pathways via ectoenzyme CD39, which leads to upregulation of the inducible

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Table 1
Studies regarding experimental and clinical strategies employing Tregs in allo-HSCT.

Strategies	Authors, yr	Mechanisms	Ref.
Experimental approaches			
Coinfusion of Tregs with stem cells	Edinger M, et al. 2003	Donor-derived Tregs could inhibit lethal GVHD via suppressing the early expansion of alloreactive donor T cells and maintain the GVT effect.	[49]
Adoptive transfer of Tregs	Nguyen VH, et al. 2008	Improving lymphoid recovery through preservation of thymus structure and protection of peripheral nodal niches.	[66]
Ex vivo-induced CD8hi Tregs	Zheng J, et al. 2013	Alleviation of GVHD through reducing alloreactive T cell proliferation and decreasing inflammatory cytokine and chemokine secretion within target organs via a CTLA-4-dependent mechanism	[56]
PTCy	Ganguly S, et al. 2014	Prevention of GVHD by PTCy was correlated with reconsitution of suppressive and epigenetically stable donor thymus–derived Tregs in secondary lymphoid organs.	[48]
Therapeutic Tregs adoptive transfer	McDonald-Hyman, et al. 2016	Treating chronic GVHD through inhibiting germinal center reactions participated by alloreactive T cells and B cells in a CXCR5-dependent manner.	[35]
TL1A-Ig and Low Dose IL-2	Copsel S, et al. 2018	'Two-pathway' expanded Tregs show increased levels of effector molecules, such as PD-1 and CTLA-4, as well as mediate enhanced in vitro suppressor activity, leading to suppression of GVHD.	[31]
Clinical approaches			
Interleukin-2	Koreth J, et al. 2011	Successful in treating chronic GVHD via *in vivo* expanding Tregs.	[81]
Treg-Tcon adoptive immunotherapy	Martelli MF, et al. 2014	Decreasing cumulative incidence of relapse through promoting immune reconstitution.	[40]
Treg-depleted DLI	Maury S, et al. 2011	Treg-depleted DLI is a safe, feasible approach that induces graft-versus-host or graft-versus-tumor effects in alloreactivity-resistant patients.	[28]

Abbreviations: Tregs = regulatory T cells; allo-HSCT = allogeneic hematopoietic stem cell transplantation; GVHD = graft-versus-host disease; GVL = graft-versus-leukemia; CTLA-4 = cytotoxic T-lymphocyte antigen 4; PTCy = post-cyclophosphamide; ALDH = Aldehyde Dehydrogenase; DLI = donor lymphocyte infusion.

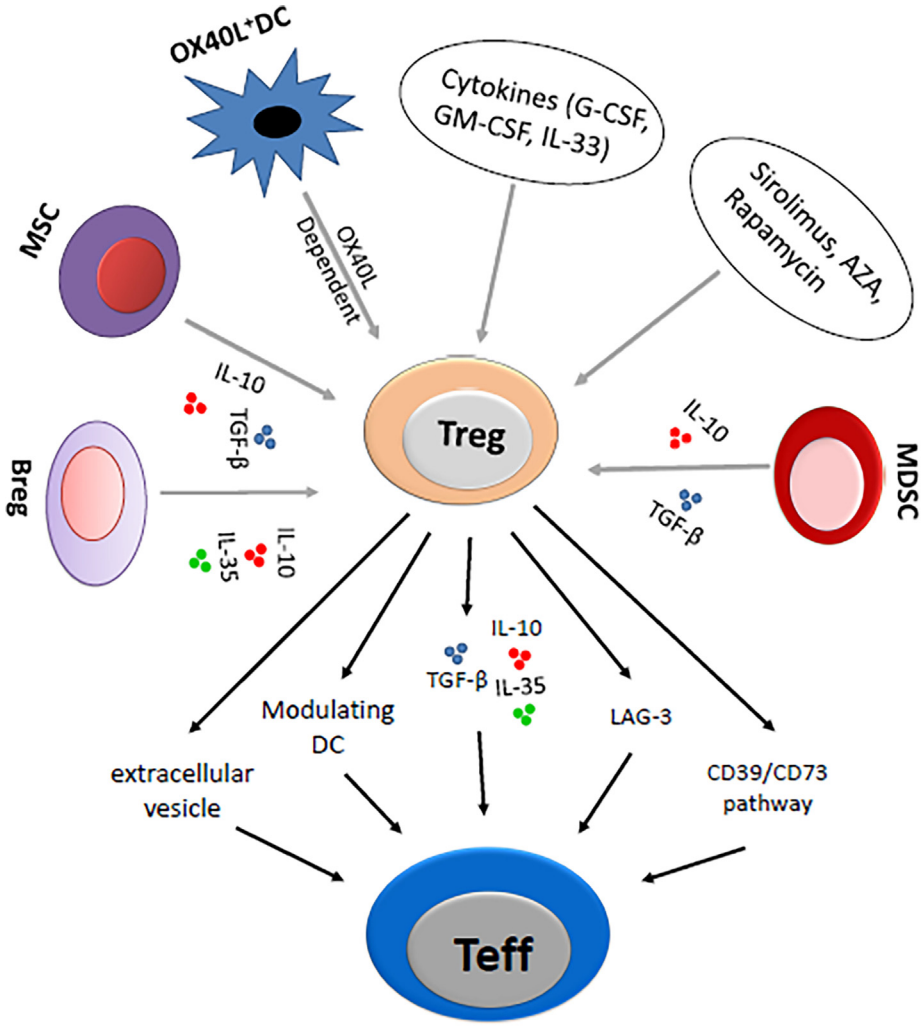

Fig. 1. Induction or expansion of Tregs by other immune cells or alternative methods. Different regulatory cells can induce Tregs via either cytokines, such as IL-10 and TGF-β, or other molecules, such as OX40L. Tregs can also be induced or expanded by a number of approaches, such as granulocyte colony-stimulating factor (G-CSF), azacitidine (AZA), and sirolimus. Tregs could regulate the function of Teff through several pathways. Abbreviations: Tregs, regulatory T cells; Bregs, regulatory B cells; MSCs, mesenchymal stem cells; MDSCs, myeloid-derived suppressor cells; DCs, dendritic cells; IL-10, interleukin-10; TGF-β, transforming grow factor-β; Teff, effective T cells.

cAMP early repressor (ICER); in turn, nuclear factor of activated T cells (NFAT) and IL-2 transcription are inhibited and apoptosis occurs due to IL-2 deprivation (Fig. 1). In addition, CD39/CD73 expressed on Tregs may also contribute to the production of adenosine which binds to A2A adenosine receptor resulting in DC and T cells inhibition. Based on the evidence that antibodies to LAG-3 could inhibit suppression by induced Tregs both *ex vivo* and *in vivo*. Moreover, the regulatory activity of $CD4^+CD25^+$ Tregs obtained from LAG-$3^{-/-}$ mice was significantly

reduced. Huang et al. [44] suggest that LAG-3, a mark of Treg, contributes to their suppressor activity. Impressively, Delgoffe et al. [45] also showed that neuropilin-1 (Nrp-1) could also maintain the stability and function of Tregs by a Nrp-1-semaphorin-4a axis. More recently, Hoeppli et al. [46] for the first time reported that *in vitro* expanded human CXCR3$^+$ Th1-Tregs with a gut-homing phenotype were functional *ex vivo* and able to delay the development of xenoGVHD *in vivo*.

Considering the risk of generalized immunosuppression of polyclonal Tregs, several researchers have investigated the roles of antigen-specific Tregs played in transplantation and autoimmune diseases [46–51]. Most of these studies showed that alloantigen-specific Tregs, enriched by engineered to express a TCR transgene or alloantigen-stimulated expansion *ex vivo*, are more potent suppressors of organ and tissue graft rejection than are polyclonal Tregs [47–51]. Some data also demonstrated that alloantigen-specific therapeutic Tregs were effectively used to prevent GVHD [48,50,51]. Overall, more and more evidence suggest that alloantigen-specific Tregs could be successfully applied in the field of transplantation and autoimmune diseases, although a lot of studies are warranted.

3. Laboratory studies of Tregs in allo-HSCT

Tregs have been confirmed to be involved in the immune tolerance induced by posttransplantation cyclophosphamide (PTCy), GVHD prevention and treatment, and enhancement of immune reconstitution after allo-HSCT.

3.1. Immune tolerance induced by PTCy via Tregs

To establish a novel haploidentical transplant protocol, PTCy is used to induce immune tolerance [52,53]. Further study indicated that aldehyde dehydrogenase (ALDH) was necessary for the ability of Tregs to survive Cy treatment in allogeneic reactions. In four separate experiments, NOD/Lt-*scid*/IL-2rγnull (NSG) mice treated with PTCy on day 3 after receiving Treg-depleted PBMC grafts had lower weights, higher GVHD scores, and died more quickly than mice treated with PTCy after receiving whole PBMC grafts ($P = 0.016$). Thus, removal of Tregs abrogated the protective effects of PTCy in this xenogeneic model. Furthermore, the NSG mice that received PTCy eventually died, suggesting that alloreactive T cells were not completely eliminated by PTCy and eventually escaped suppression to cause fatal GVHD. These findings support the idea that Tregs plays an essential role in the mechanism of GVHD prevention by PTCy [38]. In a subsequent study, Ganguly et al. [54] demonstrated that i) the prophylactic efficacy of PTCy against GVHD was dependent on donor CD4$^+$ Foxp3$^+$ Tregs and ii) PTCy treatment was associated with recovery of epigenetically stable and suppressive donor thymus-derived Tregs in secondary lymphoid organs. These findings indicate that the PTCy-mediated protection against GVHD is not singularly dependent on the depletion of donor alloreactive T cells but also requires rapid recovery of donor Tregs to initiate and maintain alloimmune regulation. Overall, the available data suggest that Tregs are the key factor in inducing immune tolerance by PTCy and establishing haploidentical HSCT based on PTCy, although the roles of other immune subsets, particularly specialized antigen-presenting cells such as dendritic cells, in the GVHD protective activity of PTCy are unknown.

3.2. Prevention and treatment of GVHD using Tregs

In 2003, Edinger et al. [55] first reported that donor-derived Tregs could not only inhibit lethal GVHD after allo-HSCT across major histocompatibility complex (MHC) class I and II barriers via suppressing the early expansion of alloreactive donor T cells in mice but could also maintain the graft-versus-tumor (GVT) effector function mediated primarily by the perforin lysis pathway. Several reports support the notion the CD4$^+$ Tregs ameliorate GVHD [27,30,36,54–61] while only a few comment on CD8$^+$ Tregs. In humanized mice, Zheng et al. [62] showed that ex vivo-induced CD8^{hi} Tregs could reduce alloreactive T cell proliferation and decrease inflammatory cytokine and chemokine secretion within target organs through a CTLA-4-dependent mechanism, leading to control of allospecific GVHD. These CD8^{hi} Tregs could also maintain general immunity and the effects of GVT simultaneously.

In addition to donor- and recipient-derived Tregs, Lim et al. [63] found that third party-derived Treg cell therapy performed equal regulation of the expansion effects on Tregs and that the effects of suppressive CD4$^+$IL-17$^+$ T helper (Th17) cells in ex vivo assays were comparable to those of the donor- and host-derived groups. In mouse models, several methods, including exogenous tumor necrosis factor receptor 2 (TNFR2) activation [64], activated protein C signals, selective TNFR2 activation, C5a/C5aR blockade [65], aurora A/JAK2 inhibition, and death receptor (DR3) signaling modulation, have been used to alleviate GVHD through a Treg-dependent mechanism.

Recently, Parmar et al. [60] found that the α-1,3-fucosyltransferase VI enzyme could significantly increase Treg surface fucosylation (66% vs 8%). Fucosylated Tregs showed prolonged periods of *in vivo* persistence and significantly ameliorated clinical GVHD and improved survival (70% vs 30%; $P < 0.0001$) of the recipient mice compared to those of mice with untreated Tregs. Currently, several alternative immune cell subsets, such as mesenchymal stem cell (MSC) and myeloid derived suppressor cells (MDSC), are known to induce Tregs (Fig. 1) [66–68]. Other strategies to enhance Treg functions include inhibition of the aryl hydrocarbon receptor (AhR antagonist/AhR (−/−)) [69], stabilization of Foxp3 by targeting JAK2 [70], and vimentin disruption [71].

Using a mouse model, McDonald-Hyman et al. [35] demonstrated that Treg infusions uniformly suppressed aGVHD, increased the Treg frequency and were effective in preventing the onset of and treating established cGVHD. The efficacy was dependent upon CXCR5-sufficient Tregs homing to and inhibiting germinal center (GC) reactions. These studies indicate that infusion of Tregs, especially those enriched for GC homing, may be desirable for cGVHD therapy. In another study, Zhao et al. [36] showed that CD103$^+$ Treg cells from chronic GVHD recipients were functional and that reinfusion of CD103$^+$ Treg cells could shift the balance between Treg cells and pathogenic T cells in chronic GVHD recipients and ameliorate ongoing disease.

Overall, preclinical experiments have confirmed the effects of Tregs in preventing and treating GVHD. Further pilot clinical studies are warranted to evaluate the efficiency and safety of Tregs in preventing and treating GVHD, as are approaches intended to enhance Treg functions and alleviate GVHD.

3.3. Enhancing immune recovery by adoptive transfer of Tregs

Using a mouse model, Nguyen et al. [72] found that adoptive transfer of Tregs resulted in the following outcomes: i) alleviation of GVHD; ii) preservation of thymic and peripheral lymph node architecture and acceleration of donor lymphoid reconstitution with a diverse TCR-Vbeta repertoire; and iii) generation of antiviral T cell responses and improved survival from murine cytomegalovirus infection. To elucidate how Tregs mediated enhancement of immune reconstitution, Bolton et al. [73] developed a lymphopenic mouse model and demonstrated that full Treg reconstitution prevented the rapid oligoclonal proliferation that gave rise to pathogenic CD4 effector T cells, while preserving the slow homeostatic form of lymphopenia-induced peripheral expansion that repopulated a diverse peripheral T cell pool. Treg-mediated CTLA-4-dependent downregulation of CD80/CD86 on DCs was critical for inhibition of the rapid proliferation and was a function of the Treg/DC ratio achieved by the reconstitution.

In summary, a number of studies using mouse models have provided insights into the roles played by Tregs not only in the induction of immune tolerance but also in enhancing immune recovery. Both of these roles can be successfully used in the clinic to improve transplant

outcomes.

4. Clinical studies of Tregs in allo-HSCT

Recently, the establishment of a novel haploidentical protocol as well as the use of Tregs to prevent GVHD, enhance immune reconstitution, and manage the relapse due to Treg deletion from donor lymphocyte infusion (DLI) have been reported by several researchers. In addition, other approaches [17,74], such as granulocyte colony-stimulating factor (G-CSF), PT/CY, azacitidine (AZA), sirolimus [75–77], everrolimus [78,79], and the combination of TL1A-Ig and low dose IL-2, have been successfully used to induce or expand Tregs for clinical use.

4.1. Establishment of a haploidentical modality

The Baltimore group confirmed the feasibility of using high-dose PTCy to overcome the HLA barrier in the clinic [3,4,8,80–82]. In vivo experiments showed that increases in $CD4^+Foxp3^+$ T cells expressing ALDH occurred after allogeneic stimulation *in vivo* and most likely contributed to bidirectional tolerance induction with PTCy [38]. Luznik et al. [80] found that the actuarial OS and event-free survival at 2 years after transplantation in 68 patients who underwent nonmyeloablative haploidentical bone marrow transplantation (BMT) with PTCy were 36% and 26%, respectively. Further studies indicated that a myeloablative conditioning regimen and G-CSF-mobilized peripheral blood allografts could be successfully applied in haploidentical transplantation with PTCy [83]. A number of studies showed that patients with hematological malignancies, such as leukemia and lymphoma, who underwent haploidentical transplantation with PTCy could achieve outcomes comparable to those who underwent HLA-matched sibling donor transplant or unrelated donor transplant [3,4,8,81,82]. Currently, a haploidentical allograft is considered an alternative source for subjects without an HLA-matched sibling donor or unrelated donor, leading to a situation in which everyone has transplant donors.

4.2. Association of Tregs with transplant outcomes

The correlation of Tregs with outcomes following allo-HSCT has been investigated extensively. Rezvani et al. [29] found that a high dose of Tregs in allografts was associated with a decreased risk of GVHD. Furthermore, the authors showed that delayed early recovery of Tregs following transplantation was associated with an increased risk of GVHD [34,59]. Zorn et al. [61] also provided evidence that subjects with active chronic GVHD had a reduced frequency of Tregs compared to those of healthy volunteers. These results suggest an association of Tregs with both acute and chronic GVHD. Further study suggested that the proportion of Tregs in allogeneic HSC grafts influenced the clinical outcome and that Treg therapies could improve allogeneic HSC transplantation [37,84].

In a recent meta-analysis, Fisher et al. [85] showed that high levels of Tregs in allografts were associated with improved overall survival [OS, HR = 0.42, P = 0.003], with a significant reduction in nonrelapse mortality (NRM, HR = 0.30, P = 0.002) and a reduced risk of acute GVHD (RR = 0.59, P = 0.01). The consistency of these findings strongly suggests that the dose of Tregs in allografts has a powerful effect on the success of allogeneic HSCT. The major challenge is to translate these findings into better selection of allografts and future donors to provide substantial improvement in allogeneic HSCT outcomes and practice.

4.3. Adoptive transfer of Tregs to prevent GVHD

In the first clinical trial [39], Tregs isolated from the third cord blood unit were expanded using anti-CD3/anti-CD28 monoclonal antibody bead stimulation, which led to a median 211-fold expansion for 17 of the 23 patients (74%) receiving the Treg infusion at the targeted dose of 30×10^5 cells/kg. All Treg products were suppressive in vitro, with median suppression at the end of the culture period of 86% (range, 39–95%) at a 1:4 ratio with an inverse correlation between post-expansion absolute number of $CD4^+/CD25^+$ cells and the level of suppression. Treg cells were infused on days +1 and +15 in 15 of the 18 patients who planned to receive two doses. The conditioning regimen included cyclophosphamide 50 mg/m^2, fludarabine 40 mg/m^2 (days −6 to −2) and 200 cGy TBI in a single fraction on day −1. GVHD prophylaxis consisted of mycophenolate mofetil and cyclosporine in the first 17 patients and sirolimus and mycophenolate mofetil in six patients following a protocol amendment. The authors found no infusional toxicities. The cumulative incidence of grade II–IV acute GVHD was decreased in the Treg group compared to that in 108 identically treated historical controls (43% vs. 61%, p = 0.05). No patients in the Treg $\geq 30 \times 10^5$/kg dose group developed chronic GVHD, which was a favorable outcome compared to an incidence of chronic GVHD of 26% in the historical controls [39]. The clinical effects of Tregs in preventing GVHD were further demonstrated by the same group [86].

In another study [22], five patients (double UCB transplant, n = 2; peripheral blood-matched unrelated donor transplant, n = 3) received UCB-Tregs (dose level = 1×10^6 cells/kg) infused one day prior to the donor graft. The ratio of conventional T cells in the donor graft was at least 10 times higher than that of the infused UCB-Tregs (ratio range, 12–356). All patients engrafted at a median of 13 days (range, 8–17 days). All evaluable patients developed ≥grade II acute GVHD, and all were alive and without evidence of disease relapse at the 1 year follow-up. No increase in chronic GVHD biomarkers (REG3a and Elafin) was observed at day 7. At the time of the last follow-up, all evaluable patients were off immune suppression.

4.4. Adoptive transfer of Tregs decreases leukemia relapse

In the haploidentical allograft modality, Martelli et al. [40] investigated whether Treg-Tcon adoptive immunotherapy prevented posttransplant leukemia relapse in a phase II study. A total body irradiation-based regimen was given to 43 adults with high-risk acute leukemia (33 with AML and 10 with ALL). The grafts contained $CD34^+$ cells (mean 9.7×10^6 cells/kg), Tregs (mean 2.5×10^6 cells/kg), and Tcons (mean 1.1×10^6 cells/kg). None of the subjects received post-transplant immunosuppression. Ninety-five percent of the patients achieved full-donor type engraftment, and 15% developed ≥grade 2 acute GVHD. After a median follow-up of 46 months, the probability of disease-free survival was 56%. The cumulative incidence of relapse was significantly better than that in historical controls (5% vs. 21%; $P = 0.03$) [40]. The authors also showed a rapid, sustained increase in peripheral blood T cell subpopulation recovery after adoptive Treg transfer. Compared with that of standard haploidentical transplantation, specific $CD4^+$ and $CD8^+$ T cells targeting opportunistic pathogens, such as Aspergillus fumigatus, Candida albicans, cytomegalovirus (CMV), adenovirus, herpes simplex virus, varicella zoster virus, and toxoplasma, emerged significantly earlier (at each time point, $P < 0.0001$). These results demonstrate that the immunosuppressive potential of Tregs can be used to suppress GVHD without loss of the benefits of graft-versus-leukemia (GVL) activity.

Overall, these careful studies of infused Tregs have established their safety, and modifications of this strategy may improve their efficacy. Further prospective, multicenter, and large-sample clinical trials are warranted to evaluate the safety and efficacy of adoptive transfer of Tregs in allo-HSCT settings.

4.5. Other strategies used for allo-SCT with a Treg-dependent mechanism

4.5.1. Interleukin-2

IL-2 has been successfully used to treat chronic GVHD [17,87,88]

and autoimmune disease, such as systemic lupus erythematosus [89]. Koreth et al. [87] performed a phase 1 dose-escalation study to determine the maximum tolerated dose of daily low-dose subcutaneous IL-2 in 29 patients with active chronic GVHD. All cases received daily IL-2 (0.3×10^6, 1×10^6, or 3×10^6 IU per square meter of body-surface area) for 8 weeks. After a 4-week hiatus, cases with a response could receive IL-2 for an extended period. The end points were safety and clinical and immunologic response. The main findings of this study include that: (i) the maximum tolerated dose of IL-2 was 1×10^6 IU per square meter. (ii) of the 23 patients who could be evaluated for response, 12 had major responses involving multiple sites. (iii) immunologic and clinical responses were sustained in cases who received IL-2 for an extended period, permitting the glucocorticoid dose to be tapered by a mean of 60% (range, 25–100). Laboratory analysis showed that the Treg:Tcon ratio increased to a median of more than five times the baseline value ($P < 0.001$) and remained elevated at 8 weeks ($P < 0.001$ for both comparisons with baseline values), then declined when the patients were not receiving IL-2. The increased numbers of Treg cells expressed the Foxp3 and could inhibit autologous conventional T cells. The results of this study suggested that the administration of daily subcutaneous low-dose IL-2 was safe in patients with active chronic GVHD, and permitted a substantial reduction in the glucocorticoid dose. Several studies have provided evidence suggesting that low-dose IL-2 administration can restore Treg homeostasis and promote expansion of this subset during the polymorphic Treg reconstitution processes after HSCT. However, several questions remain. First, the effect of IL-2 on ocular or oral chronic GVHD is unknown. Second, the optimal dose and duration of IL-2 therapy have not been defined. Third, how IL-2 fits into the cGVHD treatment algorithm remains an unanswered question. Therefore, further studies are warranted to answer these questions.

Using a mice model, Ratnasothy et al. [90] demonstrated a synergistic effect of combining K^d-specific Tregs with IL-2 therapy in prolonging skin allograft survival. The authors further showed that IL-2 could preferentially enhance the proliferation of the allospecific Tregs adoptively transferred in an antigen-dependent manner *in vivo*. The data reported by Ratnasothy et al. suggest the possibility to combine different strategies to increase Tregs function.

4.5.2. Treg-depleted donor lymphocyte infusion

Currently, the NCI recommendations list DLI as a routinely considered method for patients who have relapsed after allo-HSCT and do not have GVHD. In a multicenter phase I/II clinical trial, Maury et al. [28] reported the safety and efficacy of Treg-depleted DLI (d-DLI) therapy in 17 adult subjects with malignancy who relapsed after allo-HSCT. Only one patient received d-DLI as first-line DLI because of a persisting excess of blasts in marrow after salvage chemotherapy of acute myeloid leukemia relapse. All the other patients received an average of two standard DLIs (std-DLI) (range, 1–4) before inclusion with a mean maximal cell dose of 8×10^7 $CD3^+$ cells/kg (range, 4–18). Of those, 11 needed to receive chemotherapy for progressive disease between their last std-DLI and d-DLI. In this study, all but three patients had detectable disease at the time of d-DLI. Overall, these patients received d-DLI after a mean interval of 33 months, 14 months, and 9 months, after HSCT, relapse, and their last std-DLI, respectively. Among these patients, five cases developed acute like GVHD and one case experienced chronic like GVHD. Overall, 7 of the initial 17 patients are alive, 5 of them disease-free, with a mean follow-up for surviving patients of 24 months after their first d-DLI. Among the entire cohort, GVHD induction was found to be significantly associated with improved survival (1-year survival = 83% in the 6 patients with GVHD versus 27% in the 11 others with no GVHD induction, P = 0.035). These results offer a rational method for cellular immunotherapy in allo-HSCT settings. Nikiforow et al. [25] further confirmed that that CD25/Treg-depleted DLI could be feasible and capable of inducing graft-versus-tumor responses without excessive GVHD.

In addition to IL-2, clinical data for Treg-depleted DLI from human studies suggest that AZA has the capacity to increase circulating Tregs, especially in patients who relapse early after allo-SCT. Because Tregs are important for the control of GVHD, this phenomenon may be responsible for the low rate of GVHD seen after DLI in patients given AZA to treat relapse after allo-SCT. Therefore, additional studies are required to further characterize the immunomodulatory functions of AZA and IL-2 in the context of post-allo-SCT maintenance and salvage treatment.

5. Future directions

Over the past two decades, increasing evidence has supported the notion that Tregs can be used to induce immune tolerance in both animal models and the clinic. Several approaches, including the use of KT64/86 artificial antigen-presenting cells (aAPCs) to expand tTregs, incorporation of a single restimulation after day 12 in expansion culture [91], and CellGro DC medium-expanded Tregs [92], have been used for clinical scale expansion of Tregs. However, no studies have demonstrated successful clinical use of this technique for expanding Tregs in allo-HSCT settings. In addition, several other questions need to be answered in the future. First, although preclinical experiments and pilot clinical studies have demonstrated the feasibility of use of adoptive Tregs for GVHD prevention, randomized, controlled, prospective, clinical trials are required to evaluate the clinical efficacy and move this approach from preclinical research to standard-of care. Second, whether adoptive transfer of Tregs for the prevention and treatment of GVHD can be successfully used in different transplant modalities, especially haploidentical allografts, without compromising the anti-leukemia activity after long-term follow-up is uncertain. Third, little is known about immunological homeostasis after adoptive transfer of Tregs. Therefore, further studies are imperative. Addressing these questions based on prospective clinical trials will benefit an increasing number of patients from the successful adoptive transfer of Tregs, ultimately leading to superior transplant outcomes.

6. Conclusions

More and more evidence suggest that Tregs could be successfully used in establishing haploidentical transplant protocol, preventing GVHD, enhancing immune recovery and decreasing leukemia relapse, and treating chronic GVHD. All of these indicate that Tregs, one of the most important regulatory immune cells, might target cell in improving outcomes of allo-HSCT.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

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