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Stem cell transplantation - GvHD - Section 1

GvHD prophylaxis and treatment, new modalities

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Take-home messages

- Recent advances in understanding the pathophysiology of GvHD are being discussed.
- Certain findings in the mouse model could not be translated into the clinical application. Therefore, the advantages and shortcomings of different animal models for GvHD are being elucidated.

Introduction

Despite the advances in our understanding of the pathogenesis of acute graft-versus-host disease (aGvHD) and the prophylactic treatment with a wider array of immunosuppressive medication, about 30-50% of our patients that undergo allogeneic hematopoietic cell transplantation (alloHCT) develop grade 2-4 aGvHD.[1] aGvHD patients who are refractory to standard steroid treatment have a dismal long-term prognosis with only 5-30% overall survival.[2-4] Here we discuss different prophylactic and therapeutic modalities against aGvHD that are based on pharmacological or cellular strategies.

Current state of the art

Based on the observation that the release of pro-inflammatory cytokines is a hallmark of aGvHD many investigators have focused their work on the role of multiple cytokines in the pathophysiology of aGvHD. Highly pro-inflammatory cytokines such as IL-1β,[5] IL-6 (6,7) and TNF-α[8,9] and protective cytokines IL-10[10] and IL-11[11,12] were identified to be functionally involved in murine aGvHD. However, the findings in the mouse models are often not directly translatable into the human situation. For example in the mouse model of GvHD, IL-11 promoted T cell polarization towards a Th2 phenotype which was protective against GvHD.[11,12] However in a phase I/II double-blind, placebo-controlled study for mucositis and aGvHD prevention, recombinant human interleukin-11 was connected to a high mortality based on severe fluid retention that caused pulmonary edema.[13] This example indicates that a cytokine that was well tolerated by the mice induced severe side effects in humans. Conversely, IL-1β was shown to be a proinflammatory cytokine in some murine GvHD models,[5,14,15] while other studies in mouse models showed only a minor role for IL-1 in GvHD pathophysiology.[16] Early clinical studies using IL-1 antagonism in the therapeutic setting suggested a benefit for patients suffering from GvHD,[5,17] while a later prospective, randomized controlled trial failed to show a protective effect of IL-1 blockade in the prophylactic setting.[18] In different mouse models of GvHD, TNF-α was shown to be operational in GvHD[8,9,19] and to downmodulate the function of regulatory T cells (Treg).[20] Clinical studies using TNF-α antagonism with etanercept[21] or infliximab[22] in the therapeutic setting showed some activity against GvHD. Infliximab combined with steroids reduced GvHD severity, however the reported non-relapse mortality (NRM) was high.[22] Etanercept given as a combination therapy with inolimomab (anti-IL-2Rα) for the treatment of steroid-refractory aGvHD was connected estimated rates of 2-year overall survival of 10%.[21] Other reports on TNF-α blockade after allo-HCT showed a high incidence of fungal infections[23] and reduced GVL effects.[24] These findings are in keeping with mouse studies indicating TNF antagonism reduced GvL effects against P815 cells.[8] Another pro-inflammatory cytokine, IL-6 was shown to be responsible for aGvHD in mice.[6,7] The later prospective single-institution phase 1/2 clinical study testing the IL-6R antagonist tocilizumab for aGvHD prophylaxis showed an incidence of grade 2-4 acute GvHD in patients treated with tocilizumab at day 100 of 12% which is lower than expected.[25]

Besides blocking individual cytokines, the costimulation of T cells was recognized as a potential powerful target against aGvHD. Blockade of a major costimulatory molecule, CTLA4 was shown to reduce lethal murine GvHD.[26] CD28:CD80/86 costimulation blockade with abatacept caused low GvHD rates.[27] Another negative regulator of T cell activation namely programmed death-1 (PD-1) using checkpoint inhibition showed promising results in the mouse model[28,29] that have so far not been investigated in the clinic.

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Another potential target of GvHD are chemokines that guide the migration of T cells towards GvHD target organs.[30,31] However this strategy is seen controversial as high radiation can affect the principles of chemokine mediated tissue migration of T cells. For example CCR5 inhibition was protective against GvHD in a non-irradiated GvHD mouse model[30] while in the presence of total body irradiation (TBI) an earlier time to onset and a worsening of GvHD was observed when CCR5$^{-/-}$ T cells were applied.[32] In the GvHD prophylaxis setting a single institution phase-I trial reported that CCR5 inhibition prevents aGvHD of liver and gut before day 100.[33] T cell egress from the lymph node[34,35] and DC migration[36] were both potently inhibited by the sphingosine 1-phosphate receptor agonist FTY720 in the mouse model of GvHD. This important therapeutic concept is currently investigated by using a sphingosine 1-phosphate receptor type 1 agonist[37] in a clinical study on patients undergoing alloHCT (ClinicalTrials.gov Identifier: NCT01830010).

As aGvHD is a multifactorial disease, it is likely that inhibition of multiple layers of the disease, *e.g.* by blocking downstream signals of multiple cytokine and chemokine receptors could be more effective than classical approaches targeting an individual cytokine, chemokine or co-stimulatory molecule. Signalling of multiple cytokine receptors relies on intact Janus kinase (JAK) 1 and 2 activity (Figure 1). Based on this observation different groups could show that pharmacological inhibition of JAK1/2 reduced aGvHD in the mouse.[38,39] A later retrospective survey that included 19 stem cell transplant centers in Europe and the United States showed that the use of the JAK1/2 inhibitor ruxolitinib for steroid refractory GvHD[40] was connected to overall response rates of 81.5% (44/54) in steroid refractory aGvHD including 25 complete responses (46.3%). JAK1/2 inhibition for steroid refractory cGvHD was connected to an overall response rate of 85.4% (35/41), consistent with data in a cGvHD mouse model.[40]. Ruxolitinib is currently being investigated in a prospective trial in Germany

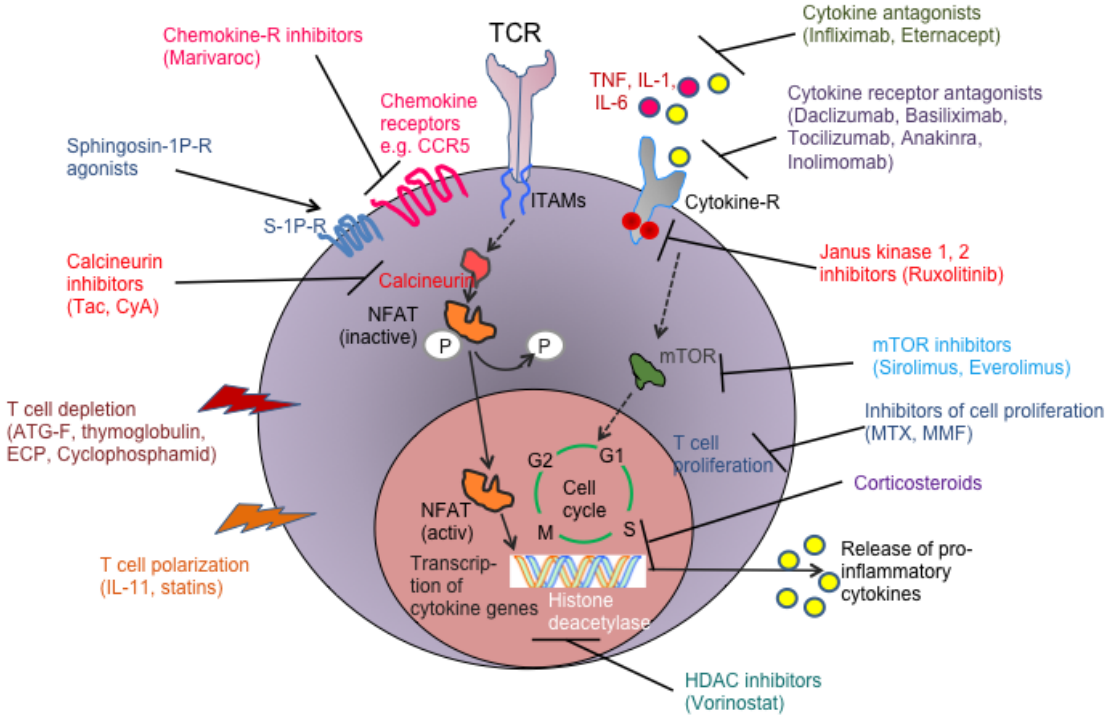

Figure 1. The different pathways for T cell activation, cytokine production and proliferation are shown in the context of their inhibitors used in GvHD prophylaxis and therapy.

(NCT02396628) and a clinical trial using the JAK1 selective inhibitor INCB39110 has begun for the treatment of GvHD (NCT02614612). Also a recent pre-clinical study indicates that topical ruxolitinib suppresses GvHD and protects skin follicular stem cells.[41] Another promising approach to reduce aGvHD in the mouse model is based on the NFkB inhibition, thereby reducing inflammatory protein production via the proteasome inhibitor bortezomib.[42] Clinical trials using a short-course, bortezomib-based GvHD prophylaxis yielded low aGvHD rates.[43,44]

Besides approaches that target the effector cells, strategies that aim at protecting target tissues were investigated. One examples is enhanced regeneration of the epithelial barrier by using a growth factor called keratinocyte growth factor (KGF).[45,46] KGF reduced aGvHD in mouse models as shown by different groups,[45,46] but the survival benefit did vary between the different reports raging from a modest improvement of the survival[45] to very potent protective effects.[46] Based on these pre-clinical data, the drug Palifermin was analyzed in a clinical study where it did not reduce aGvHD but the need for parenteral nutrition after TBI.[47,48] Another approach that aimed at enhancing epithelial regeneration via stimulation of intestinal stem cells was via R-spondin-1 which yielded promising results in the mouse model of aGvHD.[49]

The multiple approaches developed from the mouse model into a clinical application for aGvHD are summarized in Figure 1 and listed in Table 1.

We apologize to those investigators whose work could not be cited due to space restrictions.

Table 1. Translation of immunosuppressive strategies from animal models of acute GvHD into clinical trials.

Main conclusion from the preclinical model of GvHD (year)	Ref.	Main conclusion from the clinical trials (year)	Ref.
IL-11 down-regulated IL-12, and reduced aGvHD-related mortality (1998, 1999).	(11, 12)	IL-11 leads to increased mortality in patients (2002). Phase I/II double-blind, placebo-controlled study.	(13)
IL-1 blockade reduces GvHD in mice in some but not all models (1991).	(5)	IL-1 antagonist is not effective in the GvHD prophylaxis setting (2002). Phase III prospective placebo-controlled study.	(18)
TNF-α antagonism reduces GvHD (1999, 2003).	(8, 9)	Infliximab and corticosteroids are effective as initial treatment of GvHD 2009: Prospective phase III study, 2011: Retrospective analysis.	(22, 50)
IL-6 blockade reduces acute GvHD in mice (2009).	(6, 7)	Early IL-6 inhibition with tocilizumab leads to a low risk of aGvHD (2014) Phase 1/2 single institution trial.	(25).
Anti-CCR5 antibody treatment protects against aGvHD-related mortality (1999, 2003).	(30, 31)	CCR5 inhibition prevents aGvHD of liver and gut before day 100 (2012). Phase 1/2 single institution trial.	(51)
The sphingosine 1-phosphate receptor agonist FTY720 reduces GvHD (2003, 2009).	(34, 35)	Active clinical study on KRP203 in patients undergoing alloHCT (2016). Randomized, Open-label Phase 1/2 study.	(52)
CTLA4-Ig reduces lethal murine GvHD (1994).	(26)	CD28:CD80/86 costimulation blockade with abatacept leads to low GvHD rates (2013). Single-arm feasibility study.	(27)
KGF reduces but does not uniformly eliminate GvHD lethality in mice (1998, 1999).	(45, 46)	Palifermin does not reduce aGvHD severity (2012) but the need for parenteral nutrition after TBI (2013). 2012: Randomized, double-blind, placebo-controlled trial. 2013: Retrospective analysis.	(47, 48)
HDAC inhibition reduced GvHD severity in mice (2008).	(53)	Vorinostat in combination with standard GvHD prophylaxis is associated with a low incidence of severe aGvHD (2014). Phase 1/2 trial.	(54)
JAK1/2 inhibition reduces aGvHD (2014, 2015).	(38, 39)	JAK1/2 inhibition reduces aGvHD in patients refractory to multiple therapies (2015). Retrospective analysis.	(40)
Proteasome inhibition with bortezomib reduces GvHD (2004).	(42)	Short-course, bortezomib-based GvHD prophylaxis yields low aGvHD rates (2009, 2012). 2009: phase 1 trial. 2012: prospective phase I/II trial.	(43, 44)
α-GalCer reduces GvHD (2005).	(55)	RGI-2001 is tested for GvHD prevention (2016). Phase 1/2 trial.	(33)
Cyclophosphamide can induce tolerance towards skin allografts (1989)(56) and post-transplant CP reduced GvHD severity in mice (2014).	(56, 57)	Post-transplantation cyclophosphamide is effective as single-agent aGvHD Prophylaxis (2014). Open Label multi-institutional trial.	(58, 59)

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References

1. Jacobsohn DA, Vogelsang GB. Acute graft versus host disease. Orphanet J Rare Dis 2007;2:35.
2. MacMillan ML, DeFor TE, Weisdorf DJ. The best endpoint for acute GVHD treatment trials. Blood 2010;115:5412-7.
3. Westin JR, Saliba RM, De Lima M, et al. Steroid-refractory Acute GVHD: Predictors and outcomes. Adv Hematol 2011;2011:601953.
4. Martin PJ, Rizzo JD, Wingard JR, et al. First- and second-line systemic treatment of acute graft-versus-host disease: recommendations of the American Society of Blood and Marrow Transplantation. Biol Blood Marrow Transplant 2012;18:1150-63.
5. McCarthy PL, Abhyankar S i, Neben S, et al. Inhibition of interleukin-1 by an interleukin-1 receptor antagonist prevents graft-versus-host disease. Blood 1991;78:1915-8.
6. Chen X, Das R, Komorowski R, et al. Blockade of interleukin-6 signaling augments regulatory T-cell reconstitution and attenuates the severity of graft-versus-host disease. Blood 2009;114:891-900.
7. Tawara I, Koyama M, Liu C, et al. Interleukin-6 modulates graft-versus-host responses after experimental allogeneic bone marrow transplantation. Clin Cancer Res 2011;17:77-88.
8. Hill GR, Teshima T, Gerbitz A, et al. Differential roles of IL-1 and TNF-alpha on graft-versus-host disease and graft versus leukemia. J Clin Invest 1999;104:459-67.
9. Schmaltz C, Alpdogan O, Muriglan SJ, et al. Donor T cell-derived TNF is required for graft-versus-host disease and graft-versus-tumor activity after bone marrow transplantation. Blood 2003;101:2440-5.
10. Hara Mea. IL-10 is required for regulatory T cells to mediate tolerance to alloantigens in vivo. J Immunol 2001;166:3789-96.
11. Hill GR, Cooke KR, Teshima T, et al. Interleukin-11 promotes T cell polarization and prevents acute graft-versus-host disease after allogeneic bone marrow transplantation. J Clin Invest 1998;102:115-23.
12. Teshima T, Hill GR, Pan L, et al. IL-11 separates graft-versus-leukemia effects from graft-versus-host disease after bone marrow transplantation. J Clin Invest 1999;104:317-25.
13. Antin JH, Lee SJ, Neuberg D, et al. A phase I/II double-blind, placebo-controlled study of recombinant human interleukin-11 for mucositis and acute GVHD prevention in allogeneic stem cell transplantation. Bone Marrow Transplant 2002;29:373-7.
14. Jankovic D, Ganesan J, Bscheider M, et al. The Nlrp3-inflammasome regulates acute graft-versus-host disease. J Exp Med 2013;210:1899-910.
15. Chen S, Smith BA, Iype J, et al. MicroRNA-155-deficient dendritic cells cause less severe GVHD through reduced migration and defective inflammasome activation. Blood 2015;126:103-12.
16. Hanash AM, Dudakov JA, Hua G, et al. Interleukin-22 protects intestinal stem cells from immune-mediated tissue damage and regulates sensitivity to graft versus host disease. Immunity 2012;37:339-50.
17. McCarthy PL, Williams L, Harris-Bacile M, et al. A clinical phase I/II study of recombinant human interleukin-1 receptor in glucocorticoid-resistant graft-versus-host disease. Transplantation 1996;62:626-31.
18. Antin JH, Weisdorf D, Neuberg D, et al. Interleukin-1 blockade does not prevent acute graft-versus-host disease: results of a randomized, double-blind, placebo-controlled trial of interleukin-1 receptor antagonist in allogeneic bone marrow transplantation. Blood 2002;100:3479-82.
19. Stickel N, Prinz G, Pfeifer D, et al. MiR-146a regulates the TRAF6/TNF-axis in donor T cells during GvHD. Blood 2014;124:2586-95.
20. Valencia X, Stephens G, Goldbach-Mansky R, et al. TNF downmodulates the function of human CD4+CD25hi T-regulatory cells. Blood 2006;108:253-61.
21. Koreth J, Kim HT, Jones KT, et al. Efficacy, durability, and response predictors of low-dose interleukin-2 therapy for chronic graft vs. host disease. Blood 2016; pii:blood-2016-02-702852 [Epub ahead of print].
22. Couriel DR, Saliba R, de Lima M, et al. A phase III study of infliximab and corticosteroids for the initial treatment of acute graft-versus-host disease. Biol Blood Marrow Transplant 2009;15:1555-62.
23. Hahn J, Erdmann A, Grube M, et al. High incidence of invasive aspergillosis after treatment of acute GvHD with the combination of OKT3 and infliximab. Bone Marrow Transplant 2001;27:203-8.
24. Veeraputhiran M, Mangan, K. Sudden loss of the GVL effect following use of the TNF inhibitor infliximab in a chronic myelogenous leukemia patient with chronic GVHD. Bone Marrow Transplant 2010;45:1113-4.
25. Kennedy GA, Varelias A, Vuckovic S, et al. Addition of interleukin-6 inhibition with tocilizumab to standard graft-versus-host disease prophylaxis after allogeneic stem-cell transplantation: a phase 1/2 trial. Lancet Oncol 2014;13:1451-9.
26. Blazar BR, Taylor PA, Linsley PS, Vallera DA. In vivo blockade of CD28/CTLA4: B7/BB1 interaction with CTLA4-Ig reduces lethal murine graft-versus-host disease across the major histocompatibility complex barrier in mice. Blood 1994;83:3815-25.
27. Senegas-Balas F, Balas D, Verger R, et al. Immunohistochemical localization of intestinal phospholipase A2 in rat paneth cells. Histochemistry 1984;81:581-4.
28. Asakura S, Hashimoto D, Takashima S, , et al. Alloantigen expression on non-hematopoietic cells reduces graft-versus-leukemia effects in mice. J Clin Invest 2010;120:2370-8.
29. Michonneau D, Sagoo P, Breart B, et al. The PD-1 axis enforces an anatomical segregation of CTL Activity that creates tumor niches after allogeneic hematopoietic stem cell transplantation. Immunity 2016;44:143-54.
30. Murai M, Yoneyama H, Harada A, et al. Active participation of CCR5+CD8+ T lymphocytes in the pathogenesis of liver injury in graft-versus-host disease. J Clin Invest 1999;104:49-57.
31. Murai M, Yoneyama H, Ezaki T, et al. Peyer's patch is the essential site in initiating murine acute and lethal graft-versus-host reaction. Nat Immunol 2003;4:154-60.
32. Koura DT, Horan JT, Langston AA, et al. In vivo T cell costimulation blockade with abatacept for acute graft-versus-host disease prevention: a first-in-disease trial. Biol Blood Marrow Transplant 2013;11:1638-49.
33. Gonzalez M, Quezada SA, Blazar BR, et al. The balance between donor T cell anergy and suppression versus lethal graft-versus-host disease is determined by host conditioning. J Immunol 2002;169:5581-9.
34. Hashimoto D, Asakura S, Matsuoka K, Sakoda, et al. FTY720 enhances the activation-induced apoptosis of donor T cells and modulates graft-versus-host disease. Eur J Immunol 2007;37:271-81.
35. Kim Y, Sachs T, Asavaroengchai W, Bronson R, Sykes M. Graft-versus-host disease can be separated from graft-versus-lymphoma effects by control of lymphocyte trafficking with FTY720. J Clin Invest

2003;111:659-69.
36. Taylor PA, Ehrhard, MJ, Lees CJ, et al. Insights into the mechanism of FTY720 and compatibility with regulatory T cells for the inhibition of graft-versus-host disease (GVHD). Blood 2007;110:3480-8.
37. Yu X, Harden K, Gonzalez LC, et al. The surface protein TIGIT suppresses T cell activation by promoting the generation of mature immunoregulatory dendritic cells. Nat Immunol 2009;10:48-57.
38. Spoerl S, Mathew NR, Bscheider M, et al. Activity of therapeutic JAK 1/2 blockade in graft-versus-host disease. Blood 2014;123:3832-42.
39. Carniti C, Gimondi S, Vendramin A, et al. Pharmacologic inhibition of JAK1/JAK2 signaling reduces experimental murine acute GVHD while preserving GVT effects. Clin Cancer Res 2015;21:3740-9.
40. Zeiser R, Lengerke C, Spoerl S, et al. High response rates in patients treated with ruxolitinib for corticosteroid-refractory Graft-versus-host disease: analysis including 13 Transplant Centers. Bone Marrow Transplant. 2015; EBMT oral presentation.
41. Takahashi S, Hashimoto D, Hayase E, Teshima T. Topical Ruxolitinib protects LGR5+ stem cells in the hair follicle and ameliorates skin Graft-Versus-Host Disease. Biol Blood Marrow Transplant 2016; Abstract 3 ASBMT Tandem meeting 2016.
42. Kernan NA, Flomenberg N, Dupont B, O'Reilly RJ. Graft rejection in recipients of T-cell-depleted HLA-nonidentical marrow transplants for leukemia. Identification of host-derived antidonor allocytotoxic T lymphocytes. Transplantation 1987;43:842-7.
43. Goldman JM, Gale RP, Horowitz MM, et al. Bone marrow transplantation for chronic myelogenous leukemia in chronic phase. Increased risk for relapse associated with T-cell depletion. Ann Intern Med 1988;108:806-14.
44. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood 1990;75:555-62.
45. Zhao K, Zhao D, Huang D, et al. Interleukin-22 aggravates murine acute graft-versus-host disease by expanding effector T cell and reducing regulatory T cell. J Interferon Cytokine Res 2014;34:707-15.
46. Reisner Y, Kapoor N, Kirkpatrick D, et al. Transplantation for severe combined immunodeficiency with HLA-A,B,D,DR incompatible parental marrow cells fractionated by soybean agglutinin and sheep red blood cells. Blood 1983;61:341-8.
47. Filipovich AH, Vallera DA, Youle RJ, et al. Ex-vivo treatment of donor bone marrow with anti-T-cell immunotoxins for prevention of graft-versus-host disease. Lancet 1984;8375:469-72.
48. Martin PJ, Hansen JA, Buckner CD, et al. Effects of in vitro depletion of T cells in HLA-identical allogeneic marrow grafts. Blood 1985;66:664-72.
49. Beura LK, Hamilton SE, Bi K, et al. Normalizing the environment recapitulates adult human immune traits in laboratory mice. Nature. 2016;532:512-6.
50. Rager A, Frey N, Goldstein SC, et al. Inflammatory cytokine inhibition with combination daclizumab and infliximab for steroid-refractory acute GVHD. Bone Marrow Transplant. 2011;46:430-5.
51. Reshef R, Luger SM, Hexner EO, et al. Blockade of lymphocyte chemotaxis in visceral graft-versus-host disease. N Engl J Med 2012;367:135-45.
52. Guinan EC, Boussiotis VA, Neuberg D, et al. Transplantation of anergic histoincompatible bone marrow allografts. N Engl J Med 1999;340:1704-14.
53. Reddy P, Sun Y, Toubai T, et al. Histone deacetylase inhibition modulates indoleamine 2,3-dioxygenase-dependent DC functions and regulates experimental graft-versus-host disease in mice. J Clin Invest 2008;118:2562-73.
54. Choi SW, Braun T, Chang L, et al. Vorinostat plus tacrolimus and mycophenolate to prevent graft-versus-host disease after related-donor reduced-intensity conditioning allogeneic haemopoietic stem-cell transplantation: a phase 1/2 trial. Lancet Oncol 2014;1:87-95.
55. Cieri N, Camisa B, Cocchiarella F, et al. IL-7 and IL-15 instruct the generation of human memory stem T cells from naive precursors. Blood 2013;121:573-84.
56. Tichelli A, Gratwohl A, Berger C, et al. Treatment of thrombocytosis in myeloproliferative disorders with interferon alpha-2a. Blut 1989;58:15-9.
57. Casey SC, Tong L, Li Y, et al. MYC regulates the antitumor immune response through CD47 and PD-L1. Science 2016;352:227-31.
58. Kanakry CG, O'Donnell PV, Furlong T, et al. Multi-institutional study of post-transplantation cyclophosphamide as single-agent graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation using myeloablative busulfan and fludarabine conditioning. J Clin Oncol 2014;32:3497-505.
59. Luznik L, Bolaños-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood 2010;115:3224-30.