Cytokine 99 (2017) 148–153

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.elsevier.com/locate/cytokine

Profiles of pro-inflammatory cytokines in allogenic stem cell transplantation with post-transplant cyclophosphamide

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ARTICLE INFO

Keywords:
Allogeneic stem cell transplantation
Graft-versus-host-disease
Post-transplant cyclophosphamide
Biomarkers

ABSTRACT

Large number of studies was published about predictive value of cytokines for graft-versus-host disease (GVHD) after allogeneic stem cell transplantation. Recently, there has been a growing interest in GVHD prophylaxis with post-transplant cyclophosphamide (PTCy). Clinical data on the dynamics of proinflammatory cytokines with this prophylaxis is lacking. In this study, we have measured the levels of IL-17, IL-6, IL-8, IFN-γ and TNF-α in plasma on days −7, 0, +7, +14 and after engraftment in 20 patients with acute GVHD and 40 matched control patients with PTCy-based prophylaxis. Low levels of IL-8 ($p = 0.04$) on day +7 and IFN-γ ($p = 0.03$) after engraftment were associated with grade II-IV acute GVHD. The same pattern was observed for severe acute GVHD. Low IFN-γ after engraftment was also associated with increased non-relapse mortality ($p = 0.014$). No impact of cytokine levels on overall survival and relapse incidence was observed ($p > 0.05$). In conclusion, the dynamics of IL-8 and IFN-γ in GVHD patients after PTCy was different from previously reported after conventional prophylaxis.

1. Introduction

Acute graft-versus-host-disease (GVHD) remains one of the most serious complications of allogeneic hematopoietic stem cell transplantation (HSCT). The frequency of acute GVHD after HSCT using standard prophylaxis is roughly 35–50% [1]. Despite the substantial research on the treatment of acute GVHD, it remains one of the main causes of non-relapse mortality after HSCT [2,3].

The release of cytokines leading to activation of alloreactive T-cells in the early period after transplantation is an important step in the pathogenesis of acute GVHD (aGVHD). The first immunological events in GVHD occur in the first several weeks after graft transfusion [4,5]. This is the reason why many researchers try to identify predictive biomarkers for GVHD and its severity. A considerable number of studies have been focused on identifying the most predictive biomarkers. Several of them have demonstrated an increase of the soluble IL-2 receptor in patients with acute GVHD [6–9]. Elevation of TNF-alpha serum levels prior the development of GVHD was first described in 1990 [10], and the correlation between the level of TNF-alpha/TNFR1 and aGVHD was demonstrated thereafter [11–13]. Malone et al. reported increased plasma levels of IL-6 before the clinical manifestations of GVHD [14]. The correlation between the level of IFN-γ, IL-12 and GVHD corresponds to the traditional view on the development of GVHD as they trigger Th1 immune response [15–17]. IL-7, IL-15, IL-17 were also shown to have predictive value for acute GVHD [18,19]. Pro-inflammatory cytokines, including TNF-α, IL-6, IL-1β, IL-8, IL-17 and others are involved in the immune processes of chronic GVHD [20].

The majority of studies on cytokines in allogeneic HSCT were conducted with conventional GVHD prophylaxis consisting of non-specific immunosuppressive agents like calcineurin inhibitors, methotrexate, mycophenolate mofetil, antithymocyte globulin, and others. With this type of prophylaxis almost in all the studies published, higher levels of pro-inflammatory cytokines are associated with the development of acute GVHD, while lower levels indicated the success of immunosuppressive agents in abrogation of alloreactive response [21,22].

In many transplantation centers there has been a recent growing interest in the novel approach to the prevention of GVHD based on post-transplant cyclophosphamide (PTCy). A number of studies, including a study carried out by our research group, have been conducted with the use of PTCy for related, unrelated and haploidentical grafts [23–26]. The mechanism of action of PTCy, which is administered at days +3, +4 after HSCT, is based on selective depletion of alloreactive T-cells that proliferate without immunosuppression during the first three days after graft infusion [27]. In addition, PTCy facilitates rapid Treg

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http://dx.doi.org/10.1016/j.cyto.2017.08.016
Received 27 November 2016; Received in revised form 14 July 2017; Accepted 21 August 2017

expansion, compared to conventional GVHD prophylaxis [28]. Currently, there is no data, whether the dynamics of cytokines after PTCy is similar to the situation of classical GVHD prophylaxis. It is also unclear if well-known predictive biomarkers retain their value after PTCy-based prophylaxis.

Trying to answer these questions, we have conducted a pilot study to determine the dynamics of plasma cytokines in patients undergoing HSCT with PTCy as GVHD prophylaxis. For our pilot study, we have selected five cytokines, IL-17, IL-6, IL-8, IFN-γ, and TNF-α, which were shown to have predictive value for acute GVHD [10–20], and compared their levels in the patients who developed acute GVHD to those in the patients who did not.

2. Patients and transplantation procedures

Out of 192 adult patients transplanted at Pavlov First Saint Petersburg State Medical University with PTCy between 2014 and 2015, 20 cases were identified with acute GVHD and plasma samples available. These patients were matched in the ratio 1:2 to the patients who did not develop acute GVHD. The matching criteria in the order of decreasing priority were the type of the donor, the graft source, the intensity of the conditioning, diagnosis, and disease status. Thus, the study group was comprised of sixty adult patients with hematological malignancies who underwent HSCT. Exclusion criteria were the development of veno-occlusive disease and septic shock. All the patients signed informed consent forms for the collection of blood samples and the use of their personal data for the research purposes. The study was approved by the Ethical Committee of Pavlov First Saint Petersburg State Medical University.

Twenty-eight percent of the patients were grafted from a related donor, 72% – from an unrelated. The patients received either myeloablative conditioning (MAC) with oral busulfan 16 mg/kg and cyclophosphamide 100 mg/kg or reduced-intensity conditioning (RIC) with oral busulfan 8 mg/kg and fludarabine 180 mg/m^2. All the patients received PTCy-based GVHD prophylaxis. For 10/10-HLA matched unrelated donor (MUD) recipients GVHD prophylaxis consisted of the combination of cyclophosphamide 50mg/kg on days +3, +4, tacrolimus with target concentration 5–15 ng/ml starting on day +5 through day +120, and mycophenolate mofetil (MMF) 30 mg/kg, or 45 mg/kg for 8–9/10 mismatched unrelated donors (MMUD) starting day +5 through day +35. For matched related donor (MRD) with bone marrow as the graft source GVHD prophylaxis was single-agent cyclophosphamide 50 mg/kg on days +3, +4.

The patients were followed prospectively for a median of 540 days (range, 98–818 days).

3. Definitions of clinical outcomes

Time to disease relapse, aGVHD, moderate to severe chronic GVHD (cGVHD), non-relapse mortality (NRM) and overall survival (OS) was defined as the time from transplantation to the event. Incidence of aGVHD was calculated at 125 days after HSCT, and the time frame for the other outcomes was 2 years. The patients were censored at the time of last contact or a second transplantation for all outcomes. Disease relapse was defined as morphologic or cytogenetic evidence of disease with pretransplantation characteristics. Restaging evaluation, including bone marrow biopsies, was routinely performed on days +30, +60, +100, +180, and +365 post-transplant.

The Consensus Conference criteria and National Institutes of Health criteria were used for aGVHD and cGVHD grading, respectively [29,30]. The GVHD$^+$group included all acute GVHD grades (I-IV). GVHD involving two or more organs was regarded as multiorgan GVHD. All cytokines were measured before aGVHD clinical onset.

4. Blood sampling and laboratory methods

Ten mL of whole blood was obtained from the patients on days −7, 0, +7, +14. The fifth time point varied between day +21 and day +28 to represent the sample after engraftment, but before the onset of acute GVHD. Blood was collected in EDTA-containing tubes. The tubes were centrifuged for 15 min at 1000*g* and 4 °C within two hours after collection, and the plasma samples were stored in aliquots at −80 °C until the day of the assays. Five plasma biomarkers were studied: IL-17A, IL-6, IL-8, TNF-α and IFN-γ. Plasma levels were measured by ELISA using commercially available kits (Cytokine, Saint-Petersburg, Russia). All assays were performed in compliance with the protocols provided by the kit manufacturers. Concentrations were determined without knowledge of clinical data. The sensitivities of I IL-17 A, IL-6, IL-8, TNF-α, and IFN-γ were 250–10,000, 10–500, 9.75–1250, 5–250 and 50–2000 pg/ml, respectively. Samples with concentrations above the calibration curves were re-tested in dilution.

4.1. Statistical analysis

Differences in patient characteristics between GVHD+ and GVHD− groups were determined using *t*-test for age, and Fisher's exact test for all categorical variables. The Mann-Whitney *U* test was used to compare cytokine profiles according to the occurrence of GVHD. The post hoc correction for multiple group comparisons was implemented using Bonferroni method. Correlations between numerical variables were assessed using the Spearman rank test. Differences were considered statistically significant when $P < 0.05$. The cut off points for cytokine levels were determined using receiver operating curves (ROC) analysis. The survival distributions for overall survival and event-free survival were calculated using Kaplan-Meier methodology. The comparisons were made using the log-rank test. The analysis of relapse incidence and non-relapse mortality was performed using Gray test. Relapse and NRM were accounted as competing risks.

5. Results

Out of 60 patients included in the study, 20 experienced aGVHD (grades I-IV). Ten (50%) of these 20 patients developed grade I, 7 (35%) patients – grade II aGVHD, 5 (25%) patients – grade III aGVHD, 6 (30%) patients developed multiorgan aGVHD. Thirteen patients (21.6%) had chronic GVHD. Detailed patient characteristics and the analysis of differences between the groups are presented in Table 1. There was no difference between GVHD$^+$ and GVHD$^-$ groups in any of the clinical parameters. The median time of engraftment for all patients was 21 days (range 9–43). The median time to aGVHD was 30 days (range 23–92).

Neither of the cytokine levels was significantly different in the patients with grade I-IV aGVHD and the patients without GVHD. The dynamics of cytokines in these groups is presented in Fig. 1A.

However, for patients with grade II-IV aGVHD we found that low levels of IL-8 on day +7 (126.83 ± 43.794 vs 276.89 ± 310.51 pg/ml, $p = 0.04$) and IFN-γ on day +21–28 (34.70 ± 23.71 vs 60.96 ± 41.37 pg/ml, $p = 0.03$) were associated with increased risk of GVHD (Fig. 1B). The ROC analysis was performed to determine the cut off values for IL-8 – 133.56 pg/ml (AUC = 0.714) and IFN-γ – 35.94 pg/ml (AUC = 0.720). The incidence of grade II-IV aGVHD was significantly higher in the patients with the levels of cytokines lower than cut off (40% vs 5.7%, $p = 0.008$ and 43.7% vs 8.6% $p = 0.003$ for IL-8 and IFN-γ, respectively).

The same pattern was observed for the patients with grade III-IV aGVHD. Low levels of IL-8 (96.12 ± 39.79 vs 303.52 ± 346.19 pg/ml, $p = 0.008$) and IFN-γ (21.69 ± 14.78 vs 58.80 ± 39.92 pg/ml, $p = 0.012$) on day + 28 were especially predictive. The cut off values for IL-8 was 147.09 pg/ml (AUC = 0.869) and for IFN-γ – 25.71 pg/ml (AUC = 0.858). The incidence of grade II-IV aGVHD was also

Table 1
Patient characteristics.

	GVHD$^+$ (n = 20)	GVHD$^-$ (n = 40)	P value
Age	37.5 (22–56)	29.5 (18–55)	0.50
Diagnosis			0.96
AML	8 (40%)	17 (42.5%)	
ALL	4 (20%)	11 (27.5%)	
CML	4 (20%)	7 (17.5%)	
MDS	2 (10%)	2 (5%)	
HD	2 (10%)	3 (7.5%)	
Salvage	4 (18.2%)	7 (17.5%)	0.63
Donor			0.89
MRD	6 (30%)	11 (27.5%)	
MUD	14 (70%)	29 (72.5%)	
HLA matching			0.42
10/10 matched	15 (75%)	32 (80%)	
9/10 matched	5 (25%)	8 (20%)	
Conditioning			0.47
MAC	3 (15%)	8 (20%)	
RIC	17 (85%)	32 (80%)	
GVHD prophylaxis			0.89
Single agent PTCy	6 (30%)	11 (27.5%)	
PTCy + Tacro + MMF	14 (70%)	29 (72.5%)	

Age = median age in years (range); AML = acute myeloblastic leukemia; acute lymphoblastic leukemia = ALL; CML = chronic myeloid leukemia; MDS = myelodysplastic syndrome; HD = Hodgkin's disease; MRD = matched related donor; MUD = matched unrelated donor; MAC = myeloablative conditioning; RIC = reduced intensity conditioning; Tacro = tacrolimus; MMF = mycophenolate mofetil.

significantly higher in the patients with the levels of cytokines lower than cut off ($p = 0.004$ and $p = 0.0006$ for IL-8 and IFN-γ, respectively).

In additional comparative analysis in groups with multiorgan aGVHD and without GVHD we have observed lower levels of several cytokines in patients who developed GVHD. Reduced plasma levels of IL-8 (98.73 ± 39.46 vs 276.87 ± 261.34 pg/ml, $p = 0.006$ for GVHD + and GVHD-, respectively) and IFN-γ (22.69 ± 16.34 vs 59.98 ± 38.54 pg/ml, $p = 0.004$ for GVHD + and GVHD-, respectively) on day +28 were the significant risk factors for multiorgan aGVHD occurrence. The cut off values for IL-8 was 147.09 pg/ml (AUC = 0.859) and for IFN-γ – 47.05 pg/ml (AUC = 0.872). The incidence of multiorgan aGVHD was significantly higher in patients with low levels of IL-8 ($p = 0.002$) but was not predicted by IFN-γ levels.

There were no differences in clinical characteristics between subgroups of the GVHD patients that could explain the differences (supplement 1, table 1). Also neither of the other inflammatory complications, like mucositis and febrile neutropenia, were affecting the levels of IL-8 and IFN-γ at the specified time points (supplement 1, table 2).

For chronic GVHD only a higher level of IL-17 at day +28 (209.17 ± 329.59 vs 106.06 ± 210.65 pg/ml, $p = 0.037$ for the patients with and the patients without GVHD, respectively) was significantly predictive.

We also found that a lower level of IFN-γ after engraftment and a higher level of TNF-α on day 0 were associated with 2-year TRM after HSCT ($p = 0.04$ and $p = 0.014$ for IFN-γ and TNF-α, respectively). No association between the level of cytokines and overall survival, event-free survival and relapse incidence ($p > 0.05$) was observed.

6. Discussion

Although the results of more than one thousand transplants with PTCy as GVHD prophylaxis have been published [31,32], human data regarding the immunological mechanisms facilitating its efficacy is limited. In vitro studies have demonstrated that cyclophosphamide depletes both conventional T cells (Tcon) and regulatory T cells (Tregs) [33] and impairs the immunosuppressive function of the latter [34]. Thus, cyclophosphamide is proposed to be used as the potentiating agent for T cell responses and immunotherapy [35]. However, in HSCT mouse models antigenic stimulation early post transplant is proposed to cause alterations in Treg gene expression profile, resulting in high aldehyde dehydrogenase expression and resistance to PTCy of this subpopulation. The subsequent rapid expansion of Tregs is considered to be the basis of anti-GVHD activity of PTCy [36,37]. It has also been demonstrated that PTCy in mice induces expansion of myeloid-derived suppressor cells (MDSC) [38]. These two events may be linked together via the ability of MDSC population by itself to initiate expansion of Tregs [39].

Our pilot study is the first to our knowledge to have evaluated the dynamics of pro-inflammatory cytokines in patients treated with PTCy and assessed the association of their levels with GVHD. We have demonstrated that not higher, but lower levels of IL-8 and IFN-γ early post transplant are associated with the development of clinically significant acute GVHD. These results are completely different from the studies with conventional immunosuppression for GVHD prophylaxis, when elevation of these cytokines was predictive for acute GVHD [14,21]. The contradiction with the previous studies supports the preclinical data on the immunological mechanisms of PTCy. IL-8 (or CXCL8) is the chemokine that induces migration, functional control of MDSCs, and might potentiate their immunosuppressive properties [40]. IL-6 is another cytokine that augments function of MDSC [41]. The absence of significant differences of IL-6 levels in GVHD patients may be explained by low specificity of this cytokine. It is upregulated in patients with febrile neutropenia [42] and oral mucositis [43], which occur in the majority of HSCT recipients. Therefore, it is unlikely that it could be used as GVHD biomarker after PTCy. IFN-γ, on the other hand, is the key cytokine that facilitates Treg activity against experimental GVHD [44], so its low level after engraftment may represent either functional or quantitative defect of Treg population after PTCy.

The absence of IL-8 and IFN-γ predictive value in the whole cohort of acute GVHD patients is not unexpected. In the studies with classical GVHD prophylaxis, it was demonstrated that grade I acute GVHD is associated with only subtle changes of cytokine levels that can rarely result in statistically significant differences [21]. This makes further clinical studies of predictive GVHD biomarkers after PTCy even more difficult, because severe acute GVHD is observed in only a small percentage of patients [23–26], and future studies will need to enroll more than 200 transplants to comprise a representative group with severe GVHD.

Our results regarding the predictive value of IL-17 level after engraftment for the development of chronic GVHD coincide with the previous reports that this cytokine and Th17 cells play an important role, particularly in scleroderma and lung GVHD [45,46]. Thus IL-17 could be tested in larger studies to claim that its level may serve as a biomarker for chronic GVHD after PTCy.

In conclusion, in this pilot trial we have demonstrated that the dynamics of cytokines after GVHD prophylaxis with PTCy may be different from the conventional one, and well-known predictive biomarkers might not work after PTCy. Further large prospective trials are warranted to elucidate reliable biomarkers for GVHD after prophylaxis of this type.

Acknowledgements

We thank our patients, research and medical staff for making this study possible. Special thanks to V. Beklenischev who performed biobanking of plasma samples.

The reported study was funded by RFBR, according to the research project No. 16-34-60142 mol_a_dk.

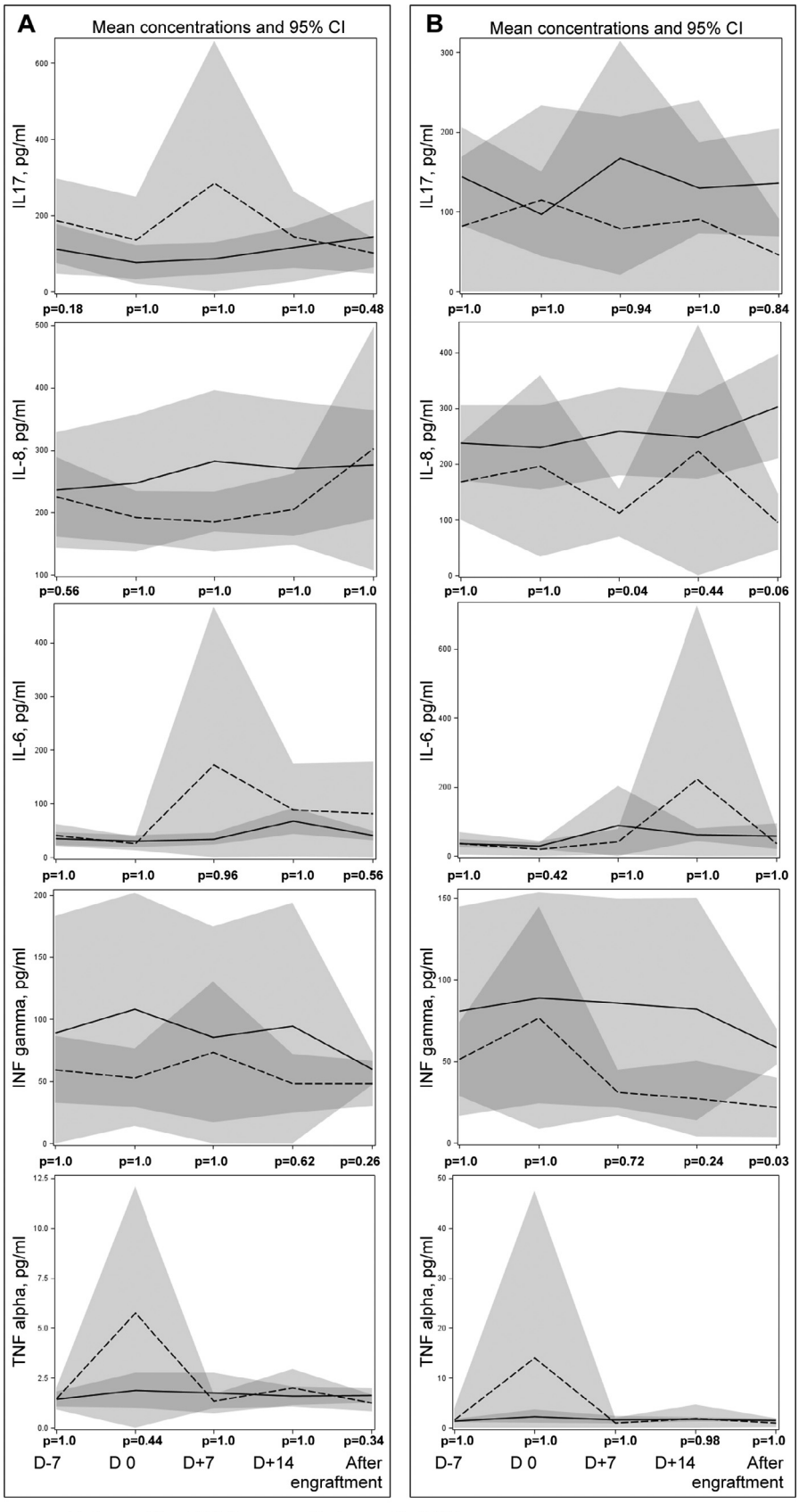

Fig. 1. Level of cytokines in patients with graft-versus-host disease. A. Grade I-IV acute GVHD. B. Grade II-IV acute GVHD. Solid lines are mean concentrations in patients with GVHD. Doted lines are mean concentrations in patients without GVHD. Grey bands represent 95% CI of concentrations. Overlapping bands represent non-significant results before post hoc correction. The p-values for each time point were calculated with Mann-Whitney test and post hoc correction for multiple comparisons.

Conflict of interest

The authors declare no conflicts of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cyto.2017.08.016.

References

[1] D.A. Jacobsohn, G.B. Vogelsang, Acute graft versus host disease, Orphanet J. Rare Dis. 2 (2007) 35, http://dx.doi.org/10.1186/1750-1172-2-35 Published online 2007 Sep 4.

[2] A. Couriel, H. Caldera, R. Champlin, K. Komanduri, Acute graft versus host disease: pathophysiology, clinical manifestations, and management, Cancer 101 (2004) 1936–1946, http://dx.doi.org/10.1002/cncr.20613.

[3] H. Goker, I.C. Haznedaroglu, N.J. Chao, Acute graft-vs-host disease: pathobiology and management, Exp. Hematol. 29 (2001) 259–277.

[4] L.M. Ball, R.M. Egeler, EBMT Paediatric Working Party. Acute GvHD: pathogenesis and classification, Bone Marrow Transplant. 41 (Suppl 2) (2008) S58–S64, http://dx.doi.org/10.1038/bmt.2008.56.

[5] S.W. Choi, J.E. Levine, J.L. Ferrara, Pathogenesis and management of graft versus host disease, Immunol. Allergy Clin. North Am. 30 (1) (2010) 75–101, http://dx.doi.org/10.1016/j.iac.2009.10.001.

[6] R. Foley, S. Couban, I. Walker, et al., Monitoring soluble interleukin-2 receptor levels in related and unrelated donor allogenic bone marrow transplantation, Bone Marrow Transplant. 21 (8) (1998) 769–773, http://dx.doi.org/10.1038/sj.bmt.1701163.

[7] T. Miyamoto, K. Akashi, S. Hayashi, et al., Serum concentration of the soluble interleukin-2 receptor for monitoring acute graft-versus-host disease, Bone Marrow Transplant. 17 (2) (1996) 185–190.

[8] C. Seidel, O. Ringdén, M. Remberger, Increased levels of syndecan-1 in serum during acute graft-versus-host disease, Transplantation 76 (2) (2003) 423–426, http://dx.doi.org/10.1097/01.TP.0000074316.76104.A5.

[9] M. Shaiegan, M. Iravani, G. Babaee, A. Ghavamzadeh, Effect of IL-18 and sIL2R on aGVHD occurrence after hematopoietic stem cell transplantation in some Iranian patients, Transpl. Immunol. 15 (3) (2006) 223–227, http://dx.doi.org/10.1016/j.trim.2005.10.002.

[10] E. Holler, H.J. Kolb, A. Möller, et al., Increased serum levels of tumor necrosis factor alpha precede major complications of bone marrow transplantation, Blood 75 (4) (1990) 1011–1016, http://dx.doi.org/10.1016/j.trim.2005.10.002.

[11] J. Cavet, P.G. Middleton, M. Segall, et al., Recipient tumor necrosis factor-alpha and interleukin-10 gene polymorphisms associate with early mortality and acute graft-versus-host disease severity in HLA-matched sibling bone marrow transplants, Blood 94 (1999) 3941–32946.

[12] A.N. Abdallah, J.M. Boiron, Y. Attia, et al., Plasma cytokines in graft vs host disease and complications following bone marrow transplantation, Hematol Cell Ther 39 (1) (1997) 27–32.

[13] S.W. Choi, C.L. Kitko, T. Braun, et al., Change in plasma tumor necrosis factor receptor 1 levels in the first week after myeloablative allogeneic transplantation correlates with severity and incidence of GVHD and survival, Blood 112 (4) (2008) 1539–1542, http://dx.doi.org/10.1182/blood-2008-02-138867.

[14] F.R. Malone, W.M. Leisenring, B.E. Storer, et al., Prolonged anorexia and elevated plasma cytokine levels following myeloablative allogeneic hematopoietic cell transplant, Bone Marrow Transplant. 40 (8) (2007) 765–772, http://dx.doi.org/10.1038/sj.bmt.1705816 Epub 2007 Aug 13.

[15] J.E. Visentainer, S.R. Lieber, L.B. Persoli, et al., Serum cytokine levels and acute graft-versus-host disease after HLA-identical hematopoietic stem cell transplantation, Exp. Hematol. 31 (2003) 1044–1050.

[16] M. Imamura, S. Hashino, H. Kobayashi, et al., Serum cytokine levels in bone marrow transplantation: synergistic interaction of interleukin-6, interferon-gamma, and tumor necrosis factor-alpha in graft-versus-host disease, Bone Marrow Transplant. 13 (1994) 745–751.

[17] H. Nakamura, K. Komatsu, M. Ayaki, et al., Serum levels of soluble IL-2 receptor, IL-12, IL-18, and IFN-gamma in patients with acute graft-versus-host disease after allogeneic bone marrow transplantation, J. Allergy Clin. Immunol. 106 (2000) S45–S50.

[18] S. Thiant, I. Yakoub-Agha, L. Magro, et al., Plasma levels of IL-7 and IL-15 in the first month after myeloablative BMT are predictive biomarkers of both acute GVHD and relapse, Bone Marrow Transplant. 45 (2010) 1546–1552, http://dx.doi.org/10.1038/bmt.2010.13.

[19] A.B. van der Waart, W.J. van der Velden, N.M. Blijlevens, H. Dolstra, Targeting the IL17 pathway for the prevention of graft-versus-host disease, Biol. Blood Marrow Transplant. 20 (6) (2014) 752–759, http://dx.doi.org/10.1016/j.bbmt.2014.02.007.

[20] J. Pidala, M. Sarwal, S. Roedder, S. Lee, Biologic markers of chronic GVHD, Bone Marrow Transplant. 49 (324–33) (2014) 1, http://dx.doi.org/10.1038/bmt.2013.97.

[21] R. Schots, L. Kaufman, I. Van Riet, et al., Proinflammatory cytokines and their role in the development of major transplant-related complications in the early phase after allogeneic bone marrow transplantation, Leukemia 17 (2003) 1150–1156, http://dx.doi.org/10.1038/sj.leu.2402946.

[22] S. Paczesny, O. Krijanovski, T. Braun, A biomarker panel for acute graft-versus-host disease, Blood 113 (2) (2009) 273–278, http://dx.doi.org/10.1182/blood-2008-07-167098.

[23] A.M. Alousi, J.E. Brammer, R.M. Saliba, et al., Phase II trial of graft-versus-host disease prophylaxis with post-transplantation cyclophosphamide after reduced-intensity busulfan/fludarabine conditioning for hematological malignancies, Biol. Blood Marrow Transplant. 21 (5) (2015) 906–912, http://dx.doi.org/10.1016/j.bbmt.2015.01.026.

[24] S.R. McCurdy, E.J. Fuchs, Comparable outcomes for hematologic malignancies after HLA-haploidentical transplantation with posttransplantation cyclophosphamide and HLA-matched transplantation, Adv Hematol. 2015 (2015) 431923, http://dx.doi.org/10.1155/2015/431923.

[25] A.M. Raiola, A. Dominietto, A. Ghiso, et al., Unmanipulated haploidentical bone marrow transplantation and posttransplantation cyclophosphamide for hematologic malignancies after myeloablative conditioning, Biol. Blood Marrow Transplant. 19 (1) (2013) 117–122, http://dx.doi.org/10.1016/j.bbmt.2012.08.014.

[26] I.S. Moiseev, O.V. Pirogova, A.L. Alyanski, et al., Graft-versus-host disease prophylaxis in unrelated peripheral blood stem cell transplantation with post-transplantation cyclophosphamide, tacrolimus, and mycophenolate mofetil, Biol. Blood Marrow Transplant. 22 (6) (2016) 1037–1042, http://dx.doi.org/10.1016/j.bbmt.2016.03.004.

[27] L. Luznik, E.J. Fuchs, High-dose, post-transplantation cyclophosphamide to promote graft-host tolerance after allogeneic hematopoietic stem cell transplantation, Immunol. Res. 47 (1–3) (2010) 65–77, http://dx.doi.org/10.1007/s12026-009-8139-0.

[28] L. Luznik, P. O'Donnell, J.F. Ephraim, Post-transplantation cyclophosphamide for tolerance induction in HLA-haploidentical BMT, Semin. Oncol. 39 (6) (2012) 683–693, http://dx.doi.org/10.1053/j.seminoncol.2012.09.005.

[29] D. Przepiorka, D. Weisdorf, P. Martin, et al., 1994 consensus conference on acute GVHD grading, Bone Marrow Transplant. 15 (1995) 825–828.

[30] A.H. Filipovich, D. Weisdorf, S. Pavletic, et al., National Institutes of health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report, Biol. Blood Marrow Transplant. 11 (2005) 945–956, http://dx.doi.org/10.1016/j.bbmt.2005.09.004.

[31] J.A. Kanakry, Y.L. Kasamon, J. Bolaños-Meade, et al., Absence of post-transplantation lymphoproliferative disorder after allogeneic blood or marrow transplantation using post-transplantation cyclophosphamide as graft-versus-host disease prophylaxis, Biol. Blood Marrow Transplant. 19 (10) (2013) 1514–1517, http://dx.doi.org/10.1016/j.bbmt.2013.07.013.

[32] A. Ruggeri, Y. Sun, M. Labopin, et al., Post-transplant cyclophosphamide versus antithymocyte-globulin as graft versus host disease prophylaxis in haploidentical transplant, Haematologica. (2016), http://dx.doi.org/10.3324/haematol.2016.151779 pii: haematol.2016.151779.

[33] I. Traverso, D. Fenoglio, S. Negrini, et al., Cyclophosphamide inhibits the generation and function of CD8(+) regulatory T cells, Hum. Immunol. 73 (3) (2012) 207–213.

[34] D. Heylmann, M. Bauer, H. Becker, et al., Human CD4+CD25+ regulatory T cells are sensitive to low dose cyclophosphamide: implications for the immune response, PLoS One 8 (12) (2013) e83384.

[35] S. Kan, S. Hazama, K. Maeda, et al., Suppressive effects of cyclophosphamide and gemcitabine on regulatory T-cell induction in vitro, Anticancer Res. 32 (12) (2012) 5363–5369.

[36] S. Ganguly, D.B. Ross, A. Panoskaltsis-Mortari, et al., Donor CD4+Foxp3+regulatory T cells are necessary for posttransplantation cyclophosphamide-mediated protection against GVHD in mice, Blood 124 (13) (2014) 2131–2141.

[37] C.G. Kanakry, S. Ganguly, M. Zahurak, et al., Aldehyde dehydrogenase expression drives human regulatory T cell resistance to posttransplantation cyclophosphamide, Sci. Transl. Med. 5 (211) (2013) 211ra157, http://dx.doi.org/10.1126/scitranslmed.3006960.

[38] J. Stokes, E.A. Hoffman, Y. Zeng, N. Larmonier, E. Katsanis, Post-transplant bendamustine reduces GvHD while preserving GvL in experimental haploidentical bone marrow transplantation, Br. J. Haematol. 174 (1) (2016) 102–116, http://dx.doi.org/10.1111/bjh.14034.

[39] Y. Luan, E. Mosheir, M.C. Menon, et al., Monocytic myeloid-derived suppressor cells accumulate in renal transplant patients and mediate CD4(+) Foxp3(+) Treg expansion, Am. J. Transplant. 13 (12) (2013) 3123–3131, http://dx.doi.org/10.1111/ajt.12461.

[40] C. Alfaro, A. Teijeira, C. Oñate, et al., Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs), Clin Cancer Res. 22 (15) (2016) 3924–3936, http://dx.doi.org/10.1158/1078-0432.CCR-15-2463.

[41] L. Cheng, J. Wang, X. Li, et al., Interleukin-6 induces Gr-1+CD11b+myeloid cells to suppress CD8+T cell-mediated liver injury in mice, PLoS One 6 (3) (2011) e17631, http://dx.doi.org/10.1371/journal.pone.0017631.

[42] N. Chaudhary, K. Kosaraju, K. Bhat, I. Bairy, A. Borker, Significance of interleukin-6 (IL-6) and C-reactive protein (CRP) in children and young adults with febrile neutropenia during chemotherapy for cancer: a prospective study, J Pediatr Hematol Oncol. 34 (8) (2012) 617–623, http://dx.doi.org/10.1097/MPH.0b013e3182677fc6.

[43] K.G. Legert, G. Tsilingaridis, M. Remberger, et al., The relationship between oral mucositis and levels of pro-inflammatory cytokines in serum and in gingival crevicular fluid in allogeneic stem cell recipients, Support. Care Cancer 23 (6) (2015) 1749–1757, http://dx.doi.org/10.1007/s00520-014-2538-z.

[44] C. Koenecke, C.W. Lee, K. Thamm, et al., IFN-γ production by allogeneic

Foxp3 + regulatory T cells is essential for preventing experimental graft-versus-host disease, J. Immunol. 189 (6) (2012) 2890–2896, http://dx.doi.org/10.4049/jimmunol.1200413.

[45] J.S. Serody, G.R. Hill, The IL-17 differentiation pathway and its role in transplant outcome, Biol. Blood Marrow Transplant. 18 (1 Suppl) (2012) S56–S61, http://dx.doi.org/10.1016/j.bbmt.2011.10.001.

[46] R.G. Resende, F. Correia-Silva Jde, T.A. Silva, et al., IL-17 genetic and immunophenotypic evaluation in chronic graft-versus-host disease, Mediators Inflamm. 2014 (2014) 571231, http://dx.doi.org/10.1155/2014/571231 Epub 2014 July 21.