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The kinetics of mRNA transforming growth factor beta1 expression and its serum concentration in graft-*versus*-host disease after allogeneic hemopoietic stem cell transplantation for myeloid leukemias

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Graft-*versus*-host disease (GVHD) is still a major complication following allogeneic hematopoietic stem cell transplantation (alloHSCT). Recent data indicates that transforming growth factor beta1 (TGF-β1) may play a role in development of GVH reaction.

Material/Methods: Forty patients with acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) were included. Quantitative real time polymerase chain reaction (RT-qPCR) was performed to assess the expression of mRNA TGF-β1. TGF-β1 serum concentration was assessed using a commercial ELISA.

Results: In all patients, a prompt decrease in TGF-β1 mRNA expression and its serum concentration was demonstrated after conditioning. In patients with acute GVHD, TGF-β1 mRNA expression and its serum concentration remained low until day +30 after transplant as compared to the day of transplant ($p<0.03$ and $p<0.006$, respectively). TGF-β1 mRNA expression and its serum concentration significantly increased on day +100 in patients who developed chronic GVHD as compared to the day of transplant ($p<0.0009$ and $p<0.02$, respectively).

Conclusions: TGF-β1 seems to be an additional regulator of donor engraftment; its low levels probably being one of the factors contributing to the development of acute GVHD. On the other hand, chronic GVHD symptoms seem to correlate with high TGF-β1 mRNA expression and its serum concentration in patients who underwent bone marrow transplantation for myeloid leukemias. Nevertheless, further studies with greater numbers of patients are needed to establish the role of TGF-β1 in graft-*versus*-host disease pathophysiology.

key words: **transforming-growth-factor beta1 • myeloid leukemia • allogeneic hematopoietic stem cell transplantation • graft-*versus*-host disease**

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Background

Graft-*versus*-host disease (GVHD) continues to be a serious complication following allogeneic hematopoietic stem cell transplantation (alloHSCT). An increasing use of unrelated and mismatched donors results in a high incidence of this complication. Acute GVHD (aGVHD) develops during the first 100 days after alloSCT, and involves the gastrointestinal tract, skin, and liver. Chronic GHVD (cGVHD) begins above 100 days after HSCT and may affect various organs.

The transforming growth factor beta1 (TGF-β1) is a major, potent regulator of immune response; it can also affect the hematopoietic cells [1,2]. Dysregulation of TGF-β pathway is associated with several malignancies [3]. TGF-β is also overproduced in autoimmune disorders and fibrosis [2,3–5]. There are some data which demonstrate that TGF-β1 may play a role in the development of graft-*versus*-host disease [6,7].

The main aim of this study was to assess TGF-β1 mRNA expression and TGF-β1 serum concentration in patients who underwent alloHSCT for myeloid leukemias. The secondary aim was to assess the correlation of the studied parameters with the occurrence of GVHD.

Material and Methods

Patients and donors

The study group comprised 40 adult patients (25 males and 15 females), median age 32 years (20–53 yrs), diagnosed with acute or chronic myeloid leukemia who underwent allogeneic bone marrow transplantation (BMT) in the Department of Hematology and Bone Marrow Transplantation in Katowice, Poland. All patients met the following inclusion criteria: first complete remission (CR1) for patients with acute myeloid leukemia (AML) and the first chronic phase (CP1) for patients with chronic myeloid leukemia (CML), all patients received BuCy (busulphan and cyclophosphamide) conditioning before transplant and cyclosporine and methotrexate were given as GVHD prophylaxis. Additionally, patients who received unrelated donor-derived stem cells were given antithymocyte globulin. Twenty-four patients were transplanted for AML and 16 patients for CML; 25 patients received their stem cells from unrelated donor whereas the donor was sibling in the remaining 15 cases. Of all patients, 20 developed the symptoms of acute GVHD, and 11 had chronic GVHD (Table 1).

Allo-BMT procedure

The myeloablative regimen consisted of busulfan 4 mg/kg/day for 4 days orally and cyclophosphamide 60 mg/kg/day for 2 days intravenously (BuCy). In case of unrelated BMT, ATG (Fresenius) at total dose of 30mg/kg i.v. from day –3 until day –1 was additionally administered. Bone marrow was a source of stem cells in all patients. A median number of transplanted nucleated cell (NC) was: 2.65 (0.4–13.6)×10^8/kg, including 2.25 (0.6–12.5)×10^6/kg of CD34+ cells and 3.5 (0.35–49.8)×10^7/kg of CD3+ cells. For GVHD prophylaxis, all patients received cyclosporine A (CsA) at a dose of 3 mg/kg i.v. from day –1 (further dosage depends on CsA plasma level and patient clinical presentation) and methotrexate (Mtx) – 15mg/m^2 on day +1 and 10 mg/m^2 on days +3 and +6. All patients were treated in reverse isolation using fungal prophylaxis and oral non-absorbable antibiotics for gastrointestinal bacterial decontamination. All patients received prophylactic acyclovir and Pneumocystis carinii prophylaxis consisting of oral trimethoprim-sulfamethoxazole. Cytomegalovirus (CMV) serological status was studied before transplantation in all donor/recipient pairs. Blood samples were obtained weekly for cytomegalovirus testing and patients were treated preemptively with ganciclovir if clinically indicated. Patients were not given prophylactic hematopoietic growth factors to enhance engraftment.

Graft-*versus*-host disease

The diagnosis of GVHD was based on physical examination and laboratory tests. Viral, allergic and drug-related causes of symptoms were ruled out. Acute GVHD was graded according to the commonly approved criteria [8]. Acute GVHD was experienced by 20 patients, and in 4 patients the symptoms were classified as severe (grades III–IV). Steroids, mainly methylprednisolone at doses ranging from 1 mg/kg to 2 mg/kg i.v. were administered for GVHD treatment. Chronic GVHD was graded according to the Seattle classification (modified) [9] and occurred in 11 patients (in 4 patients in diffuse form). Chronic GVHD symptoms were treated with methylprednisone, cyclosporine and mycofenolate.

Blood samples collection

The blood samples (10 ml of peripheral blood) were collected once a day at 4 time points: day –10 before transplant, at the day of transplant (day 0) and on days +30 and +100 after transplant. The blood samples were stored at –70°C until further investigated. The protocol was approved by the local Ethics Committee. The laboratory analysis was performed in the Department of Molecular Biology, Medical University of Silesia, Sosnowiec, Poland. All patients signed informed consent forms before entering the study.

Quantitation of the expression ratio of TGF-β1 mRNA by real-time PCR

Transcriptional activity of TGF-β1 gene and GAPDH gene (used as the endogenous control) was assessed by quantitative real time polymerase chain reaction (Q-PCR) using ABI PRISM™ 7700 (TaqMan). Total RNAs were extracted using Total RNA Prep Plus kit (AA Biotechnology), according to the manufacturer's procedure. Then, RNA was purified and treated with DNAase I on RNeasy Mini Kit columns (Qiagen). Qualitative analysis of RT-PCR products was done by electrophoresis through 1.2% agarose gel containing ethidium bromide. Specificity of the RT-PCR products (quantitative analysis) was assessed by spectrophotometric measurement using the Gene-Quant calculator (Pharmacia Biotech). Isolated material was stored at –70°C until needed for further use. RNA from an individual sample was applied for reverse transcription and amplification of TGF-β1 and GAPDH using ABI PRISM™ 7700 (TaqMan, by Applied Biosystems) according to the manufacturer's protocol. For amplification of the cDNA, specific primers were used for TGF-β1 and GAPDH. We used probes and primers from Oligo IBB PAN, Poland. The TGF-β1 TaqMan probe was 5'-CCGCTGGAGAGCCCAGCATCTGCAAAGC-3'. The TGF-β1 forward primer sequence was 5'-TGAACCGG

Table 1. Patient and donor characteristics.

		AML (n=24)	CML (n=16)
Patient age	Median (range)	32.5 years (20–53)	32 years (23–48)
	≤30 years	10	7
	>30 years	14	9
Patent sex	Female	10	5
	Male	14	11
Donor type	Related	6	4
	Unrelated	18	12
Donor age	Median (range)	36.5 years (17–52)	35 years (26–51)
	≤30 years	10	7
	>30 years	14	9
Donor sex	Female	11	6
	Male	13	10
Acute GVHD	All	11	9
	Grade I	6	7
	Grade II	1	2
	Grade III	2	–
	Grade iv	2	–
Chronic GVHD	All	7	4
	Limited	4	3
	Diffuse	3	1
Death until day +100 after BMT		4	1
Death > day +100 after BMT		5	5
Relapse until day +100 after BMT		3	–
Relapse > day +100 after BMT		2	2
Follow-up (range)		28 months (2–39 months)	

CCTTTCCTG CTTCTCATG-3' and its reverse primer sequence was 5'-GCGGAAGTCAATGTACA GCTGCCGC-3'. GAPDH TaqMan probe 5'-CAAGCTTCCCG TTCTCAGCC-3', forward 5'-GAAGGTGAAGGTCGGAGTC-3' and reverse 5'-GAAGATGGTGATGGGATTC-3' primers for internal calibration were obtained from the Rodent GAPDH Control Reagents (Applied Biosystems, USA). All the probes contained a fluorescence reporter (6-carboxyfluorescein [FAM] at the 5' end and a fluorescence quencher (6-carboxytetramethylrhodamine [TAMRA] at the 3' end. All standards and samples were assayed in triplicate. Simultaneously, amplification of a commercially available quantitative standard – β-actin, was performed (TaqMan® DNA Template Reagents Kit and β-actin Control Reagent Kit, catalogue number P/N 401970, Applied Biosystems, USA) in series of 5 times dilutions (1 to 10 000 copies of cDNA/µl). The fluorescence intensity for the series of dilutions enabled us to draw the standard curve. The threshold cycle (Ct) values were used to plot a standard curve in which Ct decreased in proportion to the log of the template copy number.

Serum TGF-β1 level measurement

Sera were pretreated with an acid-based step to activate the latent TGF-β1 to the immunoreactive form that could be detected by the immunoassay. This was achieved by acid activation and neutralization through treating samples in polypropylene test tubes with 1 N HCL and 1.2 N NaOH/0.5 M HEPES, respectively. Serum concentrations of TGF-β were assessed by using a commercial ELISA for TGF-β1 with a detection limit of 7 pg/ml (Quantikine® Human TGF-β1 Immunoassay, R&D Systems, Minneapolis, USA).

Statistical analysis

Statistical analysis was performed in accordance with the recommendations of the European Group for Blood and Marrow Transplantation (Statistical guidelines for EBMT – Labopin M., Iacobelli S., www.*ebmt.org*) and the Polish Adult Leukemia Group (PALG) [10]. A correlation between studied parameters was evaluated with the use of the Spearman

Table 2. TGF-β1 mRNA expression on consecutive study points.

mRNA TGF-β1 (copies/μg total RNA) Median (min–max)	All (n=40)	Day 0 (n=40)	Day +30 (n=40)	Day +100 (n=34)
7088 (735–82963)	Day –10	**p<0.005**	NS*	NS
1907 (157–84203)	Day 0	–	**p<0.04**	**p<0.003**
3808 (195–87720)	Day +30	–	–	NS
7531 (288–91397)	Day +100	–	–	–

***** NS – non significant.

Table 3. TGF-β1 serum concentration on consecutive study points.

TGF-β1 (pg/ml) Median (min–max)	All (n=40)	Day 0 (n=40)	Day +30 (n=40)	Day +100 (n=34)
21411 (973–44415)	Day –10	**p<0.00006**	**p<0.003**	NS
8277 (2103–37127)	Day 0	–	NS	**p<0.003**
12184 (101–28349)	Day +30	–	–	**p<0.02**
19509 (4441–37715)	Day +100	–	–	–

***** NS – non significant.

Table 4. TGF-β1 mRNA expression in patients who developed aGVHD and in patients with no aGVHD symptoms on consecutive study points.

mRNA TGF-β1 (copies/μg total RNA) Median (min–max)	No aGVHD	Day 0 (n=40)	Day +30 (n=40)
7660 (735–82963)	Day –10	NS	NS
2297 (280–84203)	Day 0	–	NS
6712 (195–87720)	Day +30		–
	aGVHD		
5728 (765–54421)	Day –10	**p<0.02**	**p<0.03**
943 (157–44392)	Day 0	–	NS
2338 (204–84606)	Day +30		–

***** NS – non significant.

rank correlation test. The significant differences between obtained values were compared using the Mann-Whitney U test and the Wilcoxon test for independent and dependent variables, respectively. To determine correlation between studied parameters on consecutive study points, the Spearman's coefficient of correlation was performed. The data were provided as median and range. P values of less than 0.05 were considered to indicate statistical significance.

Results

TGF-β1 mRNA expression on consecutive study points

A median TGF-β1 mRNA expression was 7088 copies/μg of total RNA at day –10 before transplant, dropped to 1907 copies/μg of total RNA at the day of transplant (p<0.005), and then significantly increased on days +30 (p<0.04) and +100 (p<0.003) when compared to the day of transplant (median: 3808 and 7531 copies/μg of total RNA, respectively). The results are shown in Table 2.

TGF-β1 serum concentration on consecutive study points

A median TGF-β1 serum concentration was 21411 pg/ml at day –10 before transplant, dropped to 8277 pg/ml at the day of transplant (p<0.00006); the low values (median: 12184 pg/ml) were still detected on day +30 (p<0.003) in comparison to the pretransplant period. Median TGF-β1 serum concentration significantly increased on day +100 (median: 19509 pg/ml) when compared to the day of transplant

Table 5. TGF-β1 serum concentration in patients who developed aGVHD and in patients with no aGVHD symptoms on consecutive study points.

TGF-β1 (pg/ml) Median (min – max)	No aGVHD	Day 0 (n=40)	Day +30 (n=40)
25755 (973–35516)	Day –10	**p<0.005**	NS
7916 (4416–23082)	Day 0	–	NS
12983 (3590–28349)	Day +30		–
	aGVHD		
19877 (4059–44415)	Day –10	**p<0.003**	**p<0.006**
8459 (2103–37128)	Day 0	–	NS
10371 (101–20426)	Day +30		–

***** NS – non significant.

Table 6. TGF-β1 mRNA expression in patients who developed cGVHD and in patients with no cGVHD symptoms on consecutive study points.

mRNA TGF-β1 (copies/µg total RNA) median (min – max)	No cGVHD	Day +100 (n=34)
7310 (735–82963)	Day –10	NS
2296 (172–84203)	Day 0	NS
3808 (195–87720)	Day +30	NS
5855 (288–62506)	day +100	–
	cGVHD	
5780 (794–43856)	Day –10	NS
1187 (157–9565)	Day 0	**p<0.0009**
5038 (204–84606)	Day +30	0.06
8143 (4418–91397)	Day +100	–

***** NS – non significant.

Table 7. TGF-β1 serum concentration in patients who developed aGVHD and in patients with no cGVHD symptoms on consecutive study points.

TGF-β1 (pg/ml) Median (min – max)	No cGVHD	Day +100 (n=34)
26038 (973–44415)	Day –10	NS
8641 (4526–37128)	Day 0	NS
11570 (2515–20426)	Day +30	NS
17774 (4441–37715)	Day +100	–
	cGVHD	
15588 (92622–35516)	Day –10	NS
7092 (2103–16591)	Day 0	**p<0.02**
12295 (3453–28349)	Day +30	**p<0.02**
21760 (7680–26440)	Day +100	–

***** NS – non significant.

and to the day +30 (p<0.003 and p<0.02, respectively). The results are shown in Table 3.

TGF-β1 mRNA expression and TGF-β1 serum concentration in patients who developed acute GVHD

A significant change in mRNA TGF-β1 expression was demonstrated on selected study time points in patients who developed aGVHD: a rapid decrease was observed after conditioning regimen (5728 decreased to 943 copies/µg of total RNA, p<0.02), and remained low until day +30 (2338 copies/µg of total RNA, p<0.03). On days 0 and +30 the expression of TGF-β1 was lower when compared to the patients without aGVHD (943 *vs.* 2297 and 2338 *vs.* 6712 copies/µg of total RNA, respectively), but the difference did not reach statistical significance. The results are shown in Table 4. TGF-β1 serum concentration was significantly decreased at the transplant day both in patients with and without aGVHD (25755 decreased to 7916 pg/ml in patients with no symptoms of GVHD, 19877 decreased to 8459 pg/ml in the other group, p<0.005 and p<0.003, respectively), but only in the former did it remain low on day +30 when compared to the pretransplant period (p<0.006). There was no statistically significant difference between both groups studied. The results are shown in Table 5.

TGF-β1 mRNA expression and TGF-β1 serum concentration in patients who developed chronic GVHD

A significant increase in TGF-β1 mRNA expression (up to 8143 copies/µg of total RNA), preceded by significant decrease on the day of transplant (1187 copies/µg of total RNA), was demonstrated in patients with cGVHD at day +100 (p<0.0009). There was no change in TGF-β1 mRNA expression on selected study time points in patients who did not develop cGVHD. The results are shown in Table 6. TGF-β1 serum concentration was significantly decreased at the transplant day in the group of patients with cGVHD (15588 decreased to 7092 pg/ml, p<0.002). A significant increase in TGF-β1 serum concentration (up to 21760 pg/ml) was

noted on day +100 only in patients with cGVHD ($p<0.002$) when compared to the transplant day. There was no statistically significant difference between both groups studied. The results are shown in Table 7.

The correlation between TGF-β1 mRNA expression and TGF-β1 serum concentration on consecutive study points

There was no correlation detected between these parameters, although a similar tendency was observed in both parameters – both parameters decreased after the conditioning regimen and then showed an increase up to day +100. This discrepancy might be explained by different material used for each analysis (whole blood for PCR and serum for ELISA). Moreover, the existence of latent form of TGF-β1 might also be important.

Discussion

The discovery of TGF-β1 almost 30 years ago presaged a period of discovery and insight into control of the immune system. Initial observations provided the information that TGF-β1 might not only be a mediator of neoplastic processes but also have a role in immunity. TGF-β1 is an important regulatory cytokine, involved in immunological response. It is secreted as a latent complex and can be activated by removal of the latency-associated peptide (LAP) that requires proteolytic, conformational, and/or acidic conditions [1,2]. TGF-β1 acts via TGF-β type I and II receptors. In hematopoietic cells, major targets of the TGF-βRI kinases are the receptor-regulated cytoplasmatic Smad2 and Smad3, which are recruited to the TGF-βRI through an interaction with a membrane-associated FIYE domain protein, Smad anchor for receptor activation, and which form a heterooligomeric complex incorporating the common Smad4, enabling translocation to the nucleus and formation of transcription factor complexes. TGF-β1 signaling cascades extend beyond Smad proteins to include phosphatidylinositol 3-kinase/Akt, p38 mitogen-activated protein kinase, Rho proteins, extracellular signal-regulated kinase, and stress-activated kinases [1,2]. Recent evidence indicates that the Smad pathways intersect with the Wnt signaling pathway, the interferin-gamma signal transducer and activator of transcription factor. Beyond its disparate roles in development, differentiation and tumorigenesis, TGF-β1 clearly plays a defining role as a switch factor in locoregional immune suppression. TGF-β1 is synthesized by endothelium, hemopoietic and connective tissue cells, and stored in alpha granules of platelets, which are an important source of latent TGF-β1 [1,2,5]. TGF-β1 is suspected to exert immunosuppressive and anti-inflammatory effects [1,2,5]. Its protective effects have been shown in autoimmune diabetes, inflammatory bowel disease, arthritis, acute GVHD [21], allograft rejection, and other conditions. TGF-β1 inhibits epithelial, endothelial and hematopoietic cell proliferation in normal cells, whereas in most human cancers neoplasmatic cells become resistant to TGF-β1 mainly as a result of mutations in TGF-β1 pathway [3]. TGF-β1 has a defined role in regulating hematopoiesis and is frequently dysregulated in hematological malignancies [4]. Increased TGF-β1 concentration was found in patients with advanced cancer and correlated with poor prognosis [3]. TGF-β1 is strongly associated with fibrosis [5,11]. Overexpression of TGF-β1 results in fibrosis of the kidney, liver and lung, myelofibrosis and scleroderma [5,12,13]. TGF-β1 is associated with unfavorable outcome after solid organ transplantation, causing graft fibrosis after lung transplantation or chronic rejection after liver transplantation [14,15]. The risk of fibrosis after solid organ transplantation increases when both donor and recipient have TGF-β1 polymorphisms associated with high TGF-β1 synthesis [11,14,15].

CR

To the best of our knowledge the paper we present is the first to demonstrate the kinetics of TGF-β1 changes both on the molecular and the protein level in patients who underwent HSCT for myeloid leukemias. The idea was to assess TGF-β1 mRNA expression and its serum concentration before and after BMT conditioning regimen, and then in 2 arbitrarily set post-transplant time points: on day +30 (the expected day of hemopoietic system regeneration) and on day +100 (regarded as the moment of possible chronic GVHD symptoms onset). There are no daily changes in serum TGF-β1 serum concentration; hence the only once daily measurement is valid [16].

Experimental studies indicate that high TGF-β1 levels may prevent the development of aGVHD [7,17]. Murphy et al proved that monoclonal anti-TGF-β1 antibodies significantly increase the incidence of aGVHD in mice [17]. Hirayama et al postulated that, at least in part, lymphocyte polarization towards Th2 in G-CSF-stimulated human bone marrow transplants may be responsible for overexpression of TGF-β1 and the consequently lower rate of aGVHD [6]. Moreover, G-SCF is known to increase TGF-β1 synthesis from CD4+ donor T cells [7]. This may be supported by the clinical fact that the incidence of aGVHD after G-CSF mobilized peripheral blood transplantation and unstimulated bone marrow transplantation, despite an almost 20-fold increase in T-cell content in the former, was proved to be comparable [18,19]. Some authors suggested that the qualitative changes in T-cell population induced by G-CSF may be one of the mechanisms explaining it [20]. To sum up, the production of TGF-β1 by donor T-cells seems to be one of the possible mechanisms preventing aGVHD in the early post-transplant period. Recent studies in humans seemed to confirm the hypothesis that low TGF-β1 serum concentration [21,22] and low mRNA TGF-β1 expression [6] in the early post-transplant period may be associated with the development of aGVHD. Imamura et al found a decreased TGF-β1 mRNA expression in peripheral blood mononuclear cells during aGVHD [23]. Our results showing sharp decrease in TGF-β1 serum concentration after BMT conditioning regimen and slow return to the previously observed values up to the day +100 after BMT are consistent with other published data [21]. We also showed a decrease in TGF-β1 mRNA expression and its serum concentration after myeloablative treatment, and their values remained low on day +30 when compared to the values observed on the day of transplant in the group of patients with aGVHD. We did not show this kinetics in patients with no symptoms of aGVHD, and yet the differences we found between these 2 groups of patients at consecutive time points did not reach statistical significance. We concluded that this might be associated with the low number of patients included in the study.

Recent experimental studies pointed out the differential role of TGF-β1 in aGVHD and cGVHD pathophysiology after HSCT [7]. In contrast to the potential beneficial

effects in aGVHD described above, TGF-β1 is likely to increase the risk of cGVHD. Anti-TGF-β1 antibodies may lead to lung and skin fibrosis regression in scleroderma models [24–26]. Moreover, the treatment with LAP might prevent skin fibrosis in murine sclerodermatous graft-*versus*-host disease [27]. The presence of TGF-β1 mRNA in skin samples was obtained from patients with cGVHD [28]. In a single study performed in hematological patients after HSCT high TGF-β1 serum concentration was found to correlate significantly with cGVHD symptoms [21]. In our study, elevated TGF-β1 levels (both serum concentration and mRNA expression) were observed on day +100 (in comparison to the day 0) only in patients with cGVHD. Although there was no significant difference between these 2 groups of patients at day +100, the values of TGF-β1 mRNA expression and serum concentration we obtained were higher in patients with cGVHD symptoms (8143 *vs.* 5855 copies/μg total RNA and 21760 *vs.* 17774 pg/ml, respectively). Again, further study including a greater number of patients would be needed to prove the observed tendency.

Conclusions

Our study showed kinetics changes of TGF-β1, both on molecular and protein levels, that seem to play some role in the pathophysiology of GVHD. TGF-β1 seems to be an additional regulator of donor engraftment; its low levels probably being one of the factors contributing to the development of acute GVHD. In the pathophysiology of chronic GVHD, TGF-β1 seems to be one of the factors causing fibrosis of target organs. The only problem encountered in experimental studies was the time of initiation of anti-TGF-β1 therapy [7]. Transforming growth factor-β1 seems to be a novel, additional factor in predicting the risk of graft-*versus*-host disease, and late administration of anti-TGF-β1 therapy could become a promising strategy in preventing the development of chronic graft-*versus*-host disease. Nevertheless, further studies with greater numbers of patients are needed to establish the role of TGF-β1 in graft-*versus*-host disease pathophysiology.

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