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The Role of T Regulatory Lymphocytes in Allogenic Stem Cell Transplantation Outcome

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Abstract

Background: *Haemopoietic stem cell transplantation is an effective treatment of leukemia. Mobilized PBSC are infused in the patient after a conditioning regimen consisted of chemotherapy, and/or immunosuppressive drugs aiming to target malignant cells besides creating state of immuno-suppression in order to minimize graft rejection. T-reg are CD4+CD25+FoxP3+T cells with important immuno-modulatory capabilities. They develop in thymus or induced in peripheral lymphoid tissues then exported to peripheral blood. T-reg can prevent GVHD while preserving GVL activity.*

Aim of work*: assess the role of T-reg cells in HSCT by quantifying and correlating them to HSCT outcome; engraftment or rejection and its main complication; GVHD.*

Materials and Methods: *This study was conducted on 20 patients (AML-poor risk group) and twenty healthy matched donors who were attendants to the BMT unit at Ma'adi Military Hospital and underwent an HSCT. We evaluated the correlation between Treg in reciepient PB at day 30 post-transplantation and hematopoietic engraftment represented in TLC, Neutrophil and Platelet count. We evaluated the relation between Treg cells in the recipients at day 30 after transplantation and clinical outcome by comparing Treg cells in 2 patients' groups; alive and died of GVHD.*

Results: *we found significant positive correlation between Treg and TLC and Platelets count. We found the percentage of T reg in T cell is significantly higher in alive patients than in died patients.*

Conclusion*: the balance between Treg and T eff has a great effect on the clinical outcome. When the balance is in favor of T reg, the patients acquire a good prognosis and when the balance is in favor of T cells the patients suffer from fatal GVHD.*

Introduction

Haemopoietic stem cell transplantation (HSCT) is an effective treatment of many hematological malignancies such as leukemia, lymphoma and myelodysplastic syndromes (MDS) (Colonna et al., 2011).

Donor derived bone marrow (BM) cells or mobilized peripheral blood stem cells (PBSC) are infused in the patient after a conditioning regimen consisting of chemotherapy, radiotherapy and/or immunosuppressive drugs (Noel et al., 2008) that is aimed to target malignant cells besides creating state of immuno-suppression in order to minimize graft rejection (Cao et al., 2010).

GVHD is the result of a T-cell mediated immune response and is classified as acute and chronic and overlap syndrome. Acute GVHD affect skin, gut and liver. Serious chronic GVHD is associated with loss of self-tolerance with multi-organ manifestations (Holzinger, 2010).

T regulatory lymphocytes (T-reg) are developmentally and functionally distinct T cell subpopulation. They express CD 4, CD 25 and the master switch transcription factor forkhead box P3 (FoxP3). They develop in thymus then exported to peripheral blood (PB) or induced from naïve conventional T cells in the peripheral lymphoid organs under certain condition (Linterman et al., 2011).

T-reg cells have important immuno-modulatory capabilities. They can prevent Graft versus host disease (GVHD) while preserving graft versus tumor/leukemia (GVT/L) activity (Trenado et al., 2003).

Aim of work:

To assess the role of T-reg cells in HSCT by quantifying and correlating them to HSCT outcome; engraftment or rejection and its main complication; GVHD.

Materials and Methods

This study was conducted on 40 subjects (20 patients and 20 donors) who were attendants to the BMT unit at Ma'adi Military Hospital during the period from January 2013 to December 2016 and underwent an HSCT.

All patients have been previously diagnosed AML-poor risk group by:

PB & BM morphology (Blasts > 20%), Immunophenotyping (Myeloid markers) and Karyotyping- FISH - PCR)Genetic abnormalities)

All patients have received standard AML chemotherapy and achieved CR1 then were prepared for SCT.

Patients and donors HLA typing (HLA-A, B, C and HLA-DR, DQ) and matching were performed. All donors were Matched Related Donor.

The recipients received myeloablative conditioning regimen for 5 days

The donors received G-CSF for 3 consecutive days (Filgrastim 10 mcg/kg/d) administered subcutaneously then subjected to leukapheresis.

The recipients received immunosuppressive prophylaxis for GVHD:

Patients

Patients were subjected to PB sample for flowcytometric (FCM) analysis (Immunophenotyping) and Short Tandem Repeat-Polymerase Chain Reaction (STR-PCR) besides CBC, BM microscopic examination (M/E) and other routine investigations according to the following schedule:

Time	**Sample**	**Tests**	**aim**
Day -30	PB	PCR-STR	To know the rcepient original genetics
Day -6	PB	FCM	To quantify T-cell subsets
Day Zero	PB	FCM	To quantify T-cell subsets
Day 30	BMA PB PB	M/E PCR-STR FCM	To assess engraftementto Assess chimerism To quantify T-cell subsets

Donors

Donors were subjected to PB sample according to the following schedule:

Time	Sample	Tests	
Day -30	PB	PCR-STR	To know the donors genetics
Day Zero	PB	FCM	To quantify T-cell subsets

Grafts:

1- From the stem-cell-collection pack before steroid injection into the Pack to quantify stem cells & T-cell subsets by flowcytometry.

2- From the stem-cell-collection pack after steroid injection into the Pack to quantify stem cells & T-cell subsets by flowcytometry.

1- Day -30 (1 month before donation/transplantation).

2- Day -6 (6 days before transplantation = 1 day before myeloablation).

3-Day Zero (after myeloablation and before transplantation).

4-Day 30 (1 month after transplantation = Time of chimerism evaluation).

Flowcytometric analysis of MNCs and T cell subsets (T cell, Th, Treg): Whole blood (50 µl) was combined (stained) with 5 µl flourochrome- conjugated monoclonal antibodies (mAb) of the surface markers CD4 & CD25 (FITC-labeled-CD4-mAb and PE-labeled-CD25-mAb) (BD pharmingen: BD Biosciences) in test tube and incubated for 30 minute. The stained samples are then treated with BD lysing solution which lyses erythrocytes under gentle hypotonic condition while preserving the leucocytes. After centrifugation at 3000 rpm for 5 min, the supernatant was discarded. The cells were resuspended in 250 µl fixation/permeabilization solution and incubated for 10 minutes. After centrifugation at 3000 rpm for 5 min, the supernatant was discarded. Flourochrome-conjugated mAb (5 µl) of the cytoplasmic markers; CD3 & Foxp3 (TR-conjugated-CD3-mAb and APC-conjugated -Foxp3 mAb) (Life technologies) was added and incubated for 30 minute. The cell suspension was washed once. Saline (3 ml) was added to the test tube. The cells was acquired on the BD accuri®Flowcytometry (4 colors). Events (10×104) were recorded for each sample and analysed using FlowJo software, Cell Quest software. MNCs were gated according to forward scatter (FSC) and size scatter (SSC). Then, events from the lymphocyte gate were plotted on a CD3/CD4 dot plot. CD3+CD4+cells were further subgated and plotted in CD25 versus Foxp3 dot plots. T-cells was defined as cells that are CD3+ among MNCs. T helper cells was defined as cells that are CD3+CD4+. Treg cells was defined as cells that are CD3+CD4+ CD25hiFoxp3+

Multiplex PCR amplification of Short Tandem Repeat markers and fluorescence detection for Quantitative analysis of chimerism

(STR-PCR): The method is based on a commercially available multiplex STR-PCR kit originally designed for forensic purposes (genetic fingerprinting). Nine different STR markers and the amelogenin locus are co-amplified in a single reaction. Separation of the PCR products and fluorescence detection are performed on capillary electrophoresis instruments. Calculation of donor chimerism is based on the ratio of informative donor and recipient signals.

DNA preparation/extraction/quantitation: performed within 48 h after blood collection. Samples were stored until use at −80°C. DNA was extracted from 200 µL PB or BM. Satisfactory results are obtained

with the Qiagen DNA Blood Kit (Qiagen, Hilden, Germany). Accurate DNA quantification (using standard UV-absorption at 260 nm in a DU 640-VIS Spectrophotometer; Beckman Coulter) is essential step because optimal results are only obtained within a narrow range of template DNA: 0.5-5 ng per PCR reaction. After quantification, DNA is diluted to a final conc. Of 0.125 ng/µl in 10 µl TE buffer.

PCR: is set up as described in the AmpFlSTR Profiler PCR amplification kit user manual (PE Biosystems) in a final volume of 25µl: master-mix preparation: 10.5 µl PCR buffer, 0.5 µl AmpliTaq Gold DNA polymerase and 5.5 µl of primer mix per sample (=16 µl/sample). The master-mix (15 µl) are added to 10 µl template DNA. The PCR protocol has been optimized for the PE 9600 thermocycler. After an initial incubation step at 95°C for 11 min to activate the hot-start Taq polymerase, 28 cycles are performed of the following profile: denaturation at 94°C for 1 min, annealing at 59°C for 1 min and extension at 72°C for 1 m, with a final elongation step at 60°Cfor 45 m.

Capillary electrophoresis: For fragment analysis, A 1.5 portion of the PCR reaction is mixed with 25 µl of a solution containing 24.5 µl deionized formamide and 0.5 µl Genescan ROX 500 size standard. The samples are denatured at 95°C for 3 min and chilled for at least 3 minutes in an ice-water bath. the specimens are placed in the sample rack of DNA-sequencer (ABI Prism 310 Genetic Analyzer)

Quantification of donor chimerism is performed by using the GeneMapperID v3.2 software or the Genescan 3.x software.

Results

Table 1-effect of leukapharesis on MNC: On comparing SC percentage in donor PB and SC percentage in graft, there is significant increase of MNC percentage in graft which is the main purpose of leukapheresis.

Percentage %	Donor	Graft	Independent t-test	
			t	P
MNC/All events	15.12 ± 8.69 5 – 36	32.20 ± 7.32 13 – 50	6.742	0.000
T-cells/MNC	52.90 ± 23.65 20 – 82	62.10 ± 16.01 33 – 89	1.248	0.222
Th/T	50.30 ± 14.07 32 – 73	50.60 ± 9.76 36 – 68	0.067	0.946
Treg/T	4.90 ± 4.51 0.3 – 13	3.32 ± 2.99 0.4 – 10	1.306	0.199

Table 2- effect of steroid injection into the graft: On comparing T cell subsets pattern in the graft befor and after steroid injection, there is significant decrease in T cells percentage (TCD), but the significant decrease in Treg cells percentage is more, so the balance between T cells and T reg is disturbed in favour of T cells

Percentage %		Pre steroid	Post steroid	Paired t-test	
		No.= 20	No.= 20	t	P-value
T-cells/MNC	Mean ± SD Range	62.10 ± 16.01 33 – 89	57.60 ± 14.69 32 – 70	2.784	0.021
Treg/MNC	Mean ± SD Range	2.01 ± 1.82 0.3 – 6	1.67 ± 0.85 0.34 – 2.5	2.556	0.031
Treg/T	Mean ± SD Range	3.32 ± 2.99 0.4 – 10	3.04 ± 1.45 0.5 – 4.2	2.679	0.025

Table 3- effect of Myeloablation: On comparing TLC and T cell subsets in PB of the recipient befor and after Myeloablation, there is significant decrease in all parameters

Count x 10^3/µl		Receipient day m6	Receipient day 0	Paired t-test	
				t	P-
TLC	Mean ± SD Range	8.07 ± 6.05 0.2 – 18.8	1.34 ± 0.99 0.2 – 3	4.136	0.001
T-cells	Mean ± SD Range	1.07 ± 0.93 0.08 – 3	0.03 ± 0.02 0.005 – 0.06	5.277	0.000
Th	Mean ± SD Range	0.44 ± 0.29 0.02 – 0.9	0.02 ± 0.01 0.004 – 0.04	6.257	0.000
Tc	Mean ± SD Range	0.56 ± 0.75 0.05 – 2.4	0.01 ± 0.01 0.001 – 0.03	5.136	0.000
Treg	Mean ± SD Range	0.06 ± 0.08 0 – 0.2	0.00 ± 0.00 0.001 – 0.004	3.394	0.005

Table 4- pattern of Immune reconstitution: On comparing the TLC and T cell subsets in the recipient PB before transplantation and 30 days after transplantation, there is significant increase in all parameters but the increase in Tc is the highest increase among the T cell subsets making Tc the predominant subset in the first month posttransplantation. Th increase is the lowest making Th the most delayed subset in reconstitution

Count x 10^3/µl	Receipient day 0	Receipient day 30	Paired t-test	
			t	P-
TLC	1.34 ± 0.99 0.2 – 3	4.91 ± 3.11 0.4 – 10.4	4.892	0.000
MNC	0.20 ± 0.15 0.04 – 0.58	0.53 ± 0.39 0.09 – 1.3	3.522	0.001
Tcels	0.03 ± 0.02 0.005 – 0.06	0.17 ± 0.16 0.01 – 0.55	3.884	0.000
Th	0.02 ± 0.01 0.004 – 0.04	0.08 ± 0.09 0.01 – 0.29	2.963	0.005
Tc	0.01 ± 0.01 0.001 – 0.03	0.08 ± 0.06 0 – 0.2	5.147	0.000
Treg	0.00 ± 0.00 0.001 – 0.004	0.01 ± 0.01 0 – 0.03	3.450	0.002
Percentage %				
Th/T	53 ± 15.89 13 - 83	42.30 ± 13.04 14 – 78	2.328	0.025
Tc/T	47 ± 14.32 17 - 87	57.70 ± 16.04 22 – 86	2.225	0.032
Treg/T	6.48 ± 4.06 1.6 - 12	3.77 ± 2.35 1.2 – 9.5	2.584	0.014

Table 5- Effect of Stem cell dose on hematopoiesis:

There is significant positive correlation between stem cell dose and neutrophil count in reciepient PB at day 30 posttransplantation

	Stem cell	
	r	P-value
T-cells R30	-0.414	0.111
Th R30	-0.447	0.082
Tc R30	-0.443	0.086
Treg R30	-0.455	0.077
TLC R30	-0.274	0.304
Neut R30	0.643*	0.045
Lymph R30	0.433	0.211
PLT R30	0.321	0.226

Table 6- Correlation between Tcell subsets & hematopoiesis

In reciepient PB at day 30 posttransplantation; There is significant positive correlation between all T cell subsets (T cell, Th, Tc and Treg) and TLC and Platelets count, and There is significant positive correlation between Th only and neutrophil count.

hematopoiesis	T-cells R30		Th R30		Tc R30		Treg R30	
	r	P-value	r	P-value	r	P-value	r	P-value
TLC R30	0.715**	0.000	0.747**	0.000	0.578**	0.008	0.677**	0.001
Neut R30	0.482	0.081	0.653*	0.011	0.039	0.894	0.421	0.134
Lymph R30	0.771**	0.001	0.736**	0.003	0.622*	0.017	0.593*	0.025
PLT R30	0.614**	0.004	0.644**	0.002	0.506*	0.023	0.676**	0.001

Table 7- Relation of T cell subsets to clinical outcome:

In the recipients at day 30 after transplantation; On comparing T cell subsets in the 2 patients groups (alive and died), the percentage of T reg in T cell is significantly higher in alive patients than in died patients

Percentage %	Alive	Died	Independent t-test	
	No.=8	No.=12	t	P-value
T-cells/MNC/R_30	48.38 ± 24.15 18 – 70	32.83 ± 21.87 7 – 60	1.494	0.152
Th/MNC/R_30	20.13 ± 10.93 5 – 33	18.03 ± 17.51 2 – 47	0.300	0.768
Th/T/R_30	39.50 ± 12.86 25 – 55	44.17 ± 23.30 14 – 78	-0.514	0.614
Treg/Th/R_30	11.35 ± 5.19 5 – 17	8.98 ± 5.59 3 – 20	0.954	0.353
Treg/T/R_30	4.73 ± 1.16 1.4 – 9.5	3.13 ± 1.24 1.2 – 5	2.898	0.010
Treg/MNC/R_30	2.00 ± 1.26 1 – 4	1.07 ± 0.86 0 – 3	1.982	0.063

Discussion

Haemopoietic stem cell transplantation is an effective treatment of many hematological malignancies such as leukemia, lymphoma and myelodysplastic syndromes. Donor derived BM cells or mobilized PBSC are infused in the patient after a conditioning regimen consisted of chemotherapy, radiotherapy and/or immunosuppressive drugs. Conditioning regimen is aimed to target malignant cells besides creating state of immuno- suppression in order to minimize graft rejection (Noel et al., 2008; Cao et al., 2010; Colonna et al., 2011). T-reg cells are T cells with important immuno-modulatory capabilities. They express CD 4, CD 25 and FoxP3. They develop in thymus or induced in secondary lymphoid tissues under certain conditions then exported to PB. T-reg can prevent GVHD while preserving GVT (Trenado et al., 2003).

In our study all patients have received Myelo-ablative conditioning regimen that eradicate malignant cells and offer the advantage of strong suppression of the recipient immune system which minimize graft rejection and confer good engraftement. We evaluated the effect of Myeloablation by comparing TLC and T cell subsets in PB of the recipient befor and after Myeloablation; we found significant marked reduction of all elements. This is in line with studies done by Craddock (2008). Other centres (studies) use reduced intensity conditioning or non-myeloablative regimens that often need donor lymphocyte infusions after transplantation to prevent relapse (Corradini et al., 2002) because the remaining malignant cells and T cells in the recipient may be risk factor for relapse and graft rejection.

All donors have received G-CSF as a standard mobilizing agent. PBSC have been harvested after mobilization by leukapheresis. In our study we evaluated the effect of leukapheresis on stem cells and T cell subsets by comparing SC and T cell subsets percentage in donor PB and SC percentage and T cell subsets in graft, we found significant increase of stem cells percentage in graft (1 fold) which is the main purpose of leukapheresis but the associated increase in T cell subsets was statistically non-significant. This is in line with studies done by Koca and Champlin (2008), however Cutler and Antin (2004) stated that different dose and duration of CSF or different timing of apheresis procedure led to different results. These factors could affect the speed of haematological and immunological recovery.

In an attempt to deplete T cells in the graft in our study to reduce the risk of GVHD, steroids has been injected in all grafts one hour prior to infusion into the patient. we evaluated the effect of steroid injection into the graft by comparing T cell subsets pattern in the graft befor and after steroid injection, we found significant reduction in T cells percentage, but the reduction in Treg cells percentage was significantly higher than the reduction in T cells percentage, so the balance between T cells and T reg is disturbed in favour of T cells that result in increase the risk of GVHD. Other centres use physical separation approaches like lectin agglutination (Reisner et al., 1981) or counterflow centrifugal elutriation (Noga et al., 1986). Monoclonal antibodies have been used alone (Martin et al., 1984), with homologous or rabbit

complements (Soiffer et al., 1992) as immunotoxins (Antin et al., 1991) or as immunomagnetic beads (Vartdal et al., 1987).

Yields of PBSC harvest after mobilization would be 2-4 times higher than the yield of CD34+cells collected with BM aspirate and the T cell dose delivered with PBSC grafts would be 10 times higher than the T cell dose delivered with BM grafts, thus, transfusion of PBSC graft to the patients lead to quicker hematological engraftement and immunological reconstitution, lower incidence of graft rejection or leukemia relapse and higher incidence of sever GVHD.

Among the 20 cases in our study, there was no case of rejection or relapse. This is in line with studies done by Champlin et al. (2000), Koca and Champlin. (2008), Singhal et al. (2000), Bittencourt et al. (2002), Blaise et al. (2000), Powles et al. (2000) and Brunet et al. (2001) who reported higher SC and T cell counts with PBSC grafts and subsequently better engraftement and immunological reconstitution and lower incidence of graft rejection or leukemia relapse. However Cutler et al. (2001) reported non-significant trend toward decreased relapse rate after PBSC. The discrepancy could be attributed to variables such as age, mobilization regimen, platelet counts at time of mobilization, devices used for apheresis and peaks of CD34+SC after G-CSF administration (Cottler- Fox et al., 2003).

In our study there was a good engraftement of PBSC and hematological recovery in all patients diagnosed by the significant rise of TLC and Platelets counts in patient's peripheral blood and cellular BM aspirate at day 30 post- transplantation. The dignosis of the state of full donor chimerism was made by PCR-STR to confirm that the cellular marrow is of donor origin. We evaluated the correlation between stem cell dose, T cell subsets in reciepient PB at day 30 posttransplantation and hematopoiesis represented in TLC, Neutrophil count and Platelet count in reciepient PB at day 30 posttransplantation.

We found significant significant positive correlation between stem cell dose and neutrophil count. Also, we found positive correlation between all T cell subsets (T cell, Th, Tc and Treg) and TLC and Platelets count, but there was significant positive correlation between Th only and neutrophil count. The earlier engraftement after PBSC has lead to earlier discharge from hospital, fewer transplant transfusion and total lower immediate cost associated with transplant proceduire. These findings are going with studies done by Keever-Taylor et al. (2001) and Danby et al. (2016). However, Shpall et al. (1998) stated that the Platelet recovery seems to be more sensitive to CD34+ doses than neutrophil recovery and Akpek et al. (2001) described negative correlation between T cell count and Platelet count in the context of cGVHD (the occurrence of thrombocytopenia in fatal cGVHD). The discrepancy could be attributed to difference in stem cell source, quality and quantity, also accessory cells, graft manipulation, immunosuppression caused by different conditioning regimen, degree of histocompatibility, cell composition and problems in the marrow microenvironment (Carlo-Stella, 1996).

For donor cells to accept the host environment as “self” requires that newly developing alloreactive T lymphocytes and mature donor T lymphocytes contained in the transplant inoculum be eliminated or

inactivated, and only cells tolerant to the new self be permitted in order to prevent an adverse GVH reaction. Multiple interactions between donor and host cells take place that contribute to the manifestations of this GVH reaction, leading to the picture GVHD (Platzbecker and Deeg, 2004). In our study there was 60% of the patient died from GVHD inspite of steroid injection in the graft and immunosuppressive prophylaxis and bacterial decontamination of the bowel. This is going with studies done by Sullivan et al. (1991) and Ringdén and Deeg (1997) and is different to studies by Petersdorf et al (2001) who reported probability of developing GVHD less than 30% in HLA-MSD. This discrepancy is attributed to CD4+:CD8+lymphocyte number and ratio, type of recipient immunosuppression, thymic damage, age of donor and recipient, use of prior chemotherapy and the presence or absence of infections (Krenger and Ferrara, 1996) .

In our study, we evaluated the pattern of Immune reconstitution in the first month post-transplantation by comparing the TLC and T cell subsets in the recipient PB before transplantation and 30 days after transplantation. Recovery of T lymphocytes was delayed if compared to recovery of other hemopoietic cells (neutrophils and platelets). This is going with studies done by Fukushi et al. (1990) which could be explained by the idea that T cell reconstitution is largely derived from naïve mature cells contained in the graft (decreased diversity) responding to the antigenic environment of the host. T cell is further impaired due to addition of steroids in graft and immunosuppressive prophylaxis therapy that mainly directed against T lymphocytes. Furthermore GVHD itself can directly hinder T cell reconstitution by damaging peripheral organs including thymus that is needed for T cell development from stem cell precursors. T cell reconstitution may be additionally affected by increased frequency of both CD4+ and CD8+ T cells to undergo enhanced spontaneous apoptosis in short term culture for up to one year post HSCT due to upregulation of FAS expression and a decrease in the level of bcl2/Box ratio (Hebib et al., 1999). The delay in T cell reconstitution depends on many variables such as the count of stem cells, handling of lymphocytes in the graft, prophylactic procedures to prevent GVHD, nature of hemopoietic enhancer approach and histocompatibility matching.

We found significant increase in all T cell subsets but the increase in Tc is the highest increase among the T cell subsets making Tc the predominant subset in the first month posttransplantation. This is in agreement with studies done by Schroff et al. (1982) . Th increase is the lowest, making Th to be the most delayed subset in reconstitution. Delay in CD4+ cell reconstitution is associated with immune dysfunction as CD4+ T cell are needed in most forms of immune response. This is in agreement with studies done by Douek et al. (2000).

In our study, T reg increased steadily in number in PB sample after transplantation. However as inflammation involves a complex interplay between T and non-T cell effectors, humrol responses and changes in chemokine and cytokine expression in target tissues, Therefore, although T reg are numerically elevated, this immune regulatory T cell population might not be efficient enough to suppress this inflammatory process leading to GVHD in certain patients. This is going with studies done by Clark

et al. (2004) and contradictory to Li et al. (2010). The conflicting data probably due to heterogeneous study population with respect to stem cell sources, application of TCD strategies of the graft, other variations in immunosuppression, donor cell chimerism, presence or absence of infections, further the results varied regarding the usage of different panels for phenotyping of T reg.

In our study we evaluated the relation between T cell subsets in the recipients at day 30 after transplantation and clinical outcome by comparing T cell subsets in the 2 patients groups (alive and died). We found the percentage of T reg in T cells is significantly higher in alive patients than in died patients. This indicates that the balance between Treg and T cells has a great effect on the clinical outcome. When the balance is in favor of T reg, the patients don't suffer from GVHD and acquire a good prognosis and vice versa. Lussana et al. (2017) reported similar results stating that the T reg is a hope for allogeneic SCT. That is keeping the virtue of immune response specificity if in future the investigations succeed to understand the molecular basis of immune response and the complexity of regulatory mechanisms by immunology procedures, there will be a hope to smile.

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