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**Acute but not chronic graft-versus-host disease is associated with a reduction of circulating CD4CD25CD127 regulatory T cells**

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3 **Acute but not chronic graft-versus-host disease is associated with**  
4 **a reduction of circulating CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low/-</sup> regulatory T cells**

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30 Regulatory T cells, GvHD, stem cell transplantation, reconstitution  
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## Abstract

1  
2 Defects in central and peripheral tolerance are thought to contribute to life threatening graft-  
3 versus-host disease (GvHD), a severe complication following allogeneic stem cell  
4 transplantation (SCT). Recent investigations have demonstrated regulatory T cells (Tregs) to  
5 suppress allogeneic immune reactions. Therefore, SCT patients with no or critically low  
6 numbers of Tregs may have an increased risk of GvHD. To address this hypothesis, we  
7 analyzed the recovery of CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low/-</sup> Tregs in the peripheral blood of patients  
8 who have never developed GvHD (n=6), patients who developed acute/ chronic GvHD  
9 (n=18) and patients who developed chronic GvHD without an earlier acute manifestation  
10 (n=5) every 30 days for the first six months after peripheral blood SCT (PBSCT). The number  
11 of Tregs continuously improved in acute/ chronic GvHD patients, but always remained lower  
12 than Tregs quantified in patients who never developed a GvHD. In contrast, chronic GvHD  
13 patients who did not develop acute GvHD earlier displayed significantly increased Treg cell  
14 numbers at the timepoint of chronic inflammation. These results indicate that numerically  
15 deficient Tregs following PBSCT are associated with the development of acute but not  
16 chronic GvHD.  
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## Introduction

1  
2 Allogeneic stem cell transplantation (SCT) is an effective strategy for treating haematological  
3 malignancies. However, graft-versus-host disease (GvHD) following SCT occurs in about 1/3  
4 of HLA-matched related donor transplantations and in about 2/3 of patients who are  
5 transplanted from an HLA-mismatched and/ or -matched unrelated donor [1] with a mortality  
6 rate up to 50-60% [2]. T cell depletion from the graft represents an effective approach but  
7 favors graft-rejection and aborts the graft-versus-leukemia effect. Evidence demonstrating  
8 the pivotal role that regulatory T cells (Tregs) play in GvHD development include the  
9 observation that adoptive transfer of Tregs in rodent models ameliorates this allogeneic  
10 immune response and depletion of Tregs from the stem cell graft accelerates GvHD [3-6].  
11 Initial promising results from a clinical study investigating the adoptive transfer of donor  
12 Tregs in an HLA haploidentical setting suggest that human Tregs provide beneficial effects  
13 against the development of GvHD [7]. Human studies investigating the impact of Tregs on  
14 GvHD following allogeneic SCT are conflicting, possibly due to the heterogeneity of study  
15 populations. In acute GvHD, reduced frequencies of Tregs have been shown independently  
16 of the stem cell source [8]. A study in chronic GvHD has observed increased CD4<sup>+</sup>CD25<sup>high</sup>  
17 Treg cell numbers including a high number of peripheral blood stem cell transplantations  
18 (PBSCT) [9]. In contrast, others identified decreased frequencies of circulating Tregs in  
19 chronic GvHD patients [10, 11]. However, these studies included a higher number of bone  
20 marrow (BMT) and cord blood transplantations. Here, we present the kinetic reconstitution of  
21 CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low/-</sup> Tregs during the first six months following allogeneic PBSCT in  
22 correlation to the clinical manifestation of acute and/ or chronic GvHD.  
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## Materials and Methods

### Patients

Prior to sample collection appropriate permission was given from the institutional ethics committee. Between 2007 to 2009 87 patients from the stem cell transplantation unit of Hannover Medical School were enrolled into the study. The patients donated peripheral blood samples every 4 weeks within the first 6 months following allogeneic SCT. Exclusion criteria for the immune reconstitution analysis were: missing blood samples resulting from an indisposition of the patient, withdrawal from study or death, bone marrow transplantation, incomplete donor cell chimerism and/ or signs for acute infection. Hence, presented data result from 29 patients whose characteristics are summarized in Table 1. All patients received cyclosporine (CSA) for GvHD prophylaxis. The majority of the patients developed an acute GvHD (n=18) with a high proportion of a subsequent chronic GvHD manifestation, whereas 5 patients had only signs of a chronic GvHD. Most of the GvHD patients suffered from mild to moderate acute GvHD (grade I-II) and limited chronic GvHD. Seven patients developed a severe acute GvHD (> grade II). Half of the patients who did not develop an acute and/ or chronic GvHD (n=6) received preemptive therapy with 2 mg/kg body weight prednisolone based on urine proteomic patterns highly predictive for acute GVHD [12]. Acute GvHD was staged by modified Glucksberg criteria [13] and was histologically confirmed by organ biopsies in some cases. Chronic GvHD was diagnosed by dyspigmentation and poikiloderma, lichen planus-like eruptions or sclerotic features of the skin, ulcerations and lichen-type features of the mouth as well as sicca-syndrome of the eyes and eosinophilia.

### Collection of blood samples and data analysis

Peripheral blood samples (80ml) were obtained from patients every 30 days ( $\pm$  5 days) after transplantation for six months (d30, d60, d90, d120, d150, d180). Mononucleated cells were isolated from fresh blood samples by Ficoll density gradient centrifugation. Subsequently, cells were stained with anti-human CD25 PE (Miltenyi Biotech, Germany), anti-human CD4 FITC and anti-human CD127 Alexa647 antibodies (all BD Biosciences, Germany) and

analyzed by flowcytometry (FACS Aria; BD Biosciences, Germany). Samples were first gated for lymphocytes and then gated for CD4<sup>+</sup>CD25<sup>high</sup> and CD25<sup>high</sup>CD127<sup>low/-</sup> Tregs, which resulted in a purity of > 99% as shown in Figure 1. Reconstitution of CD4<sup>+</sup> T cells and CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low/-</sup> Tregs following PBSCT was calculated using Excel 2000. The frequency of Tregs was expressed as percentage of CD4<sup>+</sup> T cells and total lymphocytes, respectively, by sequential gating on lymphocytes and CD4<sup>+</sup> T cells. Statistical analysis was performed using SigmaPlot 2000. Student's t-test was used to identify significant expression changes. P values < 0.05 were regarded statistically significant.

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## Results

### CD4<sup>+</sup> T cell and Treg cell recovery after PBSCT

In the first six months following allogeneic PBSCT, a continuously improving recovery of CD4<sup>+</sup> T cells and Tregs was observed for all 29 patients (Table 2). Quantitative differences between the clinical subgroups regarding the GvHD manifestation became statistically significant for CD4<sup>+</sup> T cell and Treg cell counts from day 90 to day 180. The lowest CD4<sup>+</sup> T cell numbers were found early after PBSCT at day 30. In this lymphopenic situation, only a very small Treg cell population circulates in the periphery. The median proportion of CD4<sup>+</sup> T cells within the total lymphocyte population increased within the six months following PBSCT, whereas the highest median percentage of Treg cells within CD4<sup>+</sup> T cells in the study population was reached at day 150. The Treg cell proportion within increasing numbers of CD4<sup>+</sup> T cells remains relatively stable with a remarkable median proportion of up to approximately 25% early after transplantation in some patients.

### Kinetic reconstitution of CD4<sup>+</sup> T cells and Tregs in immune tolerant and GvHD patients

Results from the comparative analysis of Treg cell recovery in patients with and without GvHD are presented in Table 3. In patients developing an acute GvHD, the CD4<sup>+</sup> T cell counts and their proportion within the total lymphocyte population very early after PBSCT (day 30) were higher than in immune tolerant patients. At later timepoints, the median CD4<sup>+</sup> T cell numbers were lower in the acute/ chronic GvHD patients and their proportion within the total lymphocyte population was clearly lower from day 120 onward. Continuous improvement of CD4<sup>+</sup> T cell reconstitution was only observed in immune tolerant patients within the first six months following PBSCT. In contrast, the cell numbers of circulating Tregs were continuously lower in the GvHD group in the first half year after PBSCT, whereas their proportion within the CD4<sup>+</sup> T cell population was only significantly reduced at day 30 in comparison to immune tolerant patients. Moreover, referring to the total lymphocyte population, the absolute Treg cell numbers were also clearly reduced in patients with acute GvHD at this very early timepoint, while the relative Treg cell numbers were similar in the two

1 groups (data not shown). In contrast, GvHD patients displayed an increased Treg cell  
2 proportion within the decreasing CD4<sup>+</sup> T cell population from day 60 onward (Table 3).  
3

4 Chronic GvHD patients not developing an acute manifestation after PBSCT showed nearly  
5 equal or even higher circulating Treg cell numbers than immune tolerant patients (Table 3)  
6 except at day 180, the latest timepoint evaluated. However, the clinical signs of chronic  
7 GvHD were apparent at an earlier timepoint. Interestingly, in these patients at day 30 the  
8 proportion of Tregs within the CD4<sup>+</sup> T cell population was comparable to acute/ chronic  
9 GvHD patients and lower than in immune tolerant patients, whereas later on their percentage  
10 was significantly higher. In contrast to the acute/ chronic GvHD group, chronic GvHD patients  
11 without an acute manifestation displayed higher peripheral CD4<sup>+</sup> T cell counts. Moreover, the  
12 proportion of Tregs within this numerically improving CD4<sup>+</sup> T cell population also increased  
13 (Table 3).  
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### 28 **Steroid therapy increases the percentage of Tregs**

29 In order to investigate the effect of steroid therapy on Treg recovery after PBSCT, we  
30 compared within the group of patients without clinical GvHD manifestation those patients  
31 who received preemptive prednisolone therapy (n=3) very early after SCT (< day 30) with  
32 patients that did not receive steroids (n=3). Preemptive application of prednisolone in these  
33 patients was performed because a specific proteomic expression pattern which is highly  
34 predictive for the development of GvHD was observed (see also Materials and Methods)  
35 [12]. Except for days 90 and 180, median CD4<sup>+</sup> T cell numbers were lower in the steroid  
36 treated patients compared to the untreated immune tolerant patients (Table 4). Circulating  
37 Treg cell counts were slightly lower in the steroid treated patients, whereas their median  
38 proportion within the CD4<sup>+</sup> T cell population was predominantly higher within the group of  
39 patients receiving steroids. However, probably due to the small sample size, the relative  
40 quantitative difference of Tregs within the CD4<sup>+</sup> T cell population was only statistically  
41 significant at day 30.  
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## Discussion

1  
2 Recent data from murine models implicate that regulatory T cells play a pivotal role in the  
3 maintenance of immune tolerance and may suppress graft-versus-host disease in humans  
4 following allogeneic SCT. To determine whether Tregs ameliorate GvHD, several studies  
5 were performed to quantify this regulatory T cell subpopulation after SCT in correlation to the  
6 incidence of GvHD. Currently, the results on Treg reconstitution are conflicting, probably due  
7 to heterogeneous study populations with respect to the stem cell source, application of T cell  
8 depletion strategies of the graft, other variations of the immunosuppression, donor cell  
9 chimerism and/ or active infections. Therefore, only patients without any variations of these  
10 parameters have been included in the present study. Furthermore, the recent studies varied  
11 regarding the phenotyping of Tregs: For example, Clark et al. quantified CD4<sup>+</sup>CD25<sup>high</sup> T cells  
12 [9], thus probably including activated T cells. The intracellular marker and master  
13 transcription factor for Tregs, FoxP3, which was used in later studies [8, 11], was meanwhile  
14 also identified in human non-regulatory CD4<sup>+</sup>Foxp3<sup>+</sup> T cells [14]. However, expression of IL-  
15 7Rα (CD127) has been established as a useful marker for discriminating CD4<sup>+</sup>CD25<sup>+</sup> Treg  
16 cells from activated T cells [15]. Therefore, we monitored the kinetic reconstitution of Tregs  
17 by phenotyping CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low/-</sup> T cells every 4 weeks during the first six months  
18 after allogeneic PBSCT.

19  
20 We observed a reduction of absolute and relative Treg cell numbers in acute GvHD patients  
21 as compared to patients without clinical signs of GvHD. Thus, our results confirm former  
22 reports in which different Treg phenotyping strategies were applied, including T cell depleted  
23 SCT regimens as well [8, 10, 16]. In our study, the proportion of Tregs in the decreasing  
24 CD4<sup>+</sup> T cell population improved markedly in GvHD patients during the first six months after  
25 allogeneic SCT. In contrast, chronic GvHD patients, who exhibited only a limited clinical  
26 manifestation in our analysis, had not only a relative increase within the CD4<sup>+</sup> T cell  
27 population, but also significantly higher circulating Treg cell numbers around day 100 in  
28 comparison to the no GvHD group. This numerical induction of Tregs could only be  
29 monitored in chronic GvHD patients, who did not previously develop an acute GvHD.

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However, chronic inflammation involves a complex interplay between T and non T cell effectors, humoral responses and changes in chemokine and cytokine expression in target tissues. Therefore, although Tregs are numerically elevated in chronic GvHD, this immune regulatory T cell population might not be efficient enough to suppress this chronic inflammatory process.

Consistent with our data, Clark et al. described elevated Treg cell levels in chronic GvHD patients [9]. This is the only available study which is comparable to our analysis regarding the stem cell source for transplantation as PBSCT was performed in the majority of the patients. However, whereas Clark et al. included mostly patients suffering from extensive chronic GvHD in their study [9], the patients in our study predominantly presented a limited manifestation. In another study that included a high number of bone marrow and cord blood transplantations, extensive chronic GvHD was associated with a reduction of CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low/-</sup> Tregs [10]. Zorn et al. also included mainly BM transplanted patients (n=26 of 30 patients) and also observed a reduced frequency of FoxP3<sup>+</sup>CD4<sup>+</sup>CD25<sup>+</sup> Tregs in patients with predominantly mild chronic GvHD [11].

Our analysis provides very preliminary data that suggest a possible role of glucocorticoid therapy in the numerical induction of regulatory T cells. Recently, we have shown that specific urine proteomic patterns are highly predictive for the development of GvHD [12]. The kinetic analysis of Treg reconstitution in a small cohort of these patients without clinical signs of GvHD indicates an induction of Tregs at day 30, probably resulting from preemptive steroid therapy. Consequently, conclusions regarding Treg recovery in patients with acute and chronic GvHD should be handled with care. Future research should clarify whether changes in Treg frequencies in GvHD patients are drug induced or disease related.

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## **Competing interests**

The author(s) declare having no competing interests.

## Figure legends

### Figure 1. Phenotypical analysis of CD4<sup>+</sup>CD25<sup>high</sup>CD127<sup>low/-</sup> regulatory T cells.

Unfractionated peripheral blood mononuclear cells (PBMCs) were stained with anti-human CD25 PE (Miltenyi Biotech), anti-human CD4 FITC and anti-human CD127 AlexaFluor 647 (BD Biosciences) and have been analyzed by FACS. Cells were gated on lymphocytes via their forward- and side-scatter properties. The resulting CD4<sup>+</sup>CD25<sup>high</sup> regulatory T cell population was expressing CD127<sup>low/-</sup> and showed a purity >99%. Representative data of one individual are presented here.

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Figure1

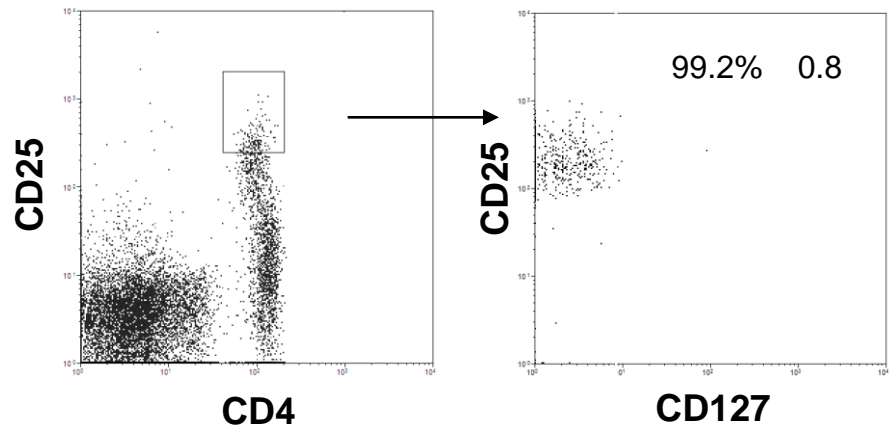


Figure 1

**Table 1. Patients' characteristics**

Pts no.	age	sex	diagnosis	conditioning protocol	donor age	donor sex	donor type	CD34 <sup>+</sup> T cells kg/ bw [x10 <sup>6</sup> ]	CD3 <sup>+</sup> T cells [ x 10 <sup>8</sup> ]	nuclear cells [ x 10 <sup>8</sup> ]	GvHD
1	54	m	MDS	Flamsa/TBI/ATG	20	m	MUD	4.20	3.9	9.60	acute
2	55	f	MDS	CIARA/TBI/ATG	44	m	MUD	5.90	3.17	16.80	acute
3	56	m	AML	Bu/Cy/ATG	46	m	MMUD	10.60	3.51	6.80	acute
4	47	m	AML	Bu/Cy	41	m	MRD	3.88	n.a.	8.74	acute
5	36	m	AML	Flamsa/TBI/ATG	38	m	MMUD	12.70	298	7.65	acute/chronic
6	31	m	ALL	TBI/VP-16/ATG	28	f	MRD	4.70	n.a.	5.64	acute/chronic
7	45	m	MPS	Flu/Bu/ATG	48	f	MRD	10.40	n.a.	5.85	acute/chronic
8	27	f	CML	Bu/Cy/ATG	26	f	MUD	9.30	3.38	10.99	acute/chronic
9	46	m	MPS	Flu/Bu/ATG	37	m	MUD	5.50	4.09	10.30	acute/chronic
10	61	m	AML	Flamsa/TBI/ATG	27	m	MMUD	9.20	6.00	14.00	acute/chronic
11	50	m	MDS	Flamsa/TBI/ATG	45	f	MRD	2.50	n.a.	3.10	acute/chronic
12	58	m	MDS	CIARA/TBI/ATG	46	m	MRD	4.12	2.51	8.32	acute/chronic
13	21	m	AML	Flu/TBI/ATG	21	m	MUD	10.50	2.41	8.57	acute/chronic
14	32	f	ALL	TBI/VP-16	29	f	MRD	7.40	3.02	6.75	acute/chronic
15	62	m	AML	Flamsa/TBI/ATG	25	f	MMUD	5.35	6.07	12.30	acute/chronic
16	51	m	MDS/MPS	Flu/Bu/ATG	47	f	MRD	11.40	n.a.	15.32	acute/chronic
17	50	m	AML	Bu/Cy/ATG	42	m	MUD	5.19	3.01	8.14	acute/chronic
18	39	m	MDS	CIARA/TBI/ATG	42	m	MUD	4.12	5.57	9.05	acute/chronic
19	42	m	AML	CIARA/TBI/ATG	27	f	MMUD	5.10	3.88	13.02	chronic
20	54	m	AML	Flu/TBI	53	m	MRD	7.20	4.63	10.30	chronic
21	52	m	MDS	CIARA/TBI/ATG	39	m	MUD	8.10	n.a.	9.70	chronic
22	61	m	MDS	CIARA/TBI/ATG	35	m	MMUD	7.00	5.93	11.20	chronic
23	30	m	ALL	TBI/Cy/ATG	37	m	MMUD	8.16	6.03	14.00	chronic
24	58	f	Plasmozytom	Flu/Melph/ATG	60	m	MRD	5.90	2.97	7.50	no GvHD
25	58	f	MDS	Flamsa/TBI/ATG	44	m	MUD	5.90	4.05	12.48	no GvHD
26	65	f	MDS/MPS	Flamsa/TBI/ATG	61	f	MRD	4.20	7.23	16.73	no GvHD
27	66	m	MDS	ClaraC/TBI/ATG	68	m	MRD	5.70	2.61	8.76	no GvHD*
28	35	f	AML	Flu/TBI/ATG	20	f	MUD	13.00	3.45	8.43	no GvHD*
29	44	f	MPS	Flu/Bu/ATG	55	f	MUD	6.00	2.27	15.90	no GvHD*

Pts indicates patients, AML acute myeloid leukemia, ALL acute lymphatic leukemia, MDS myeloid dysplastic syndrome, MPS myeloproliferative syndrome, CML chronic myeloid leukemia, TBI total body irradiation, Flu fludarabine, BU busulfan, Cy cyclophosphamide, ATG antithymocytes globuline, ClaraC clofarabine/ Ara-C, Flamsa fludarabine AraC amsacrin, Melphalan, VP-16 etoposide, MRD matched related donor, MUD matched unrelated donor, MMUD mismatched unrelated donor, n.a not applicable, \* patients that received preemptive steroid therapy with 2mg/kg body weight prednisolone, GvHD graft-versus-host disease.



Table 2. Absolute and relative T cell reconstitution following allogeneic PBSCT.

median	d30	d60	d90	d120	d150	d180
<b>CD4<sup>+</sup>/μl</b>	7.9 (0.8-73.2)	19.2 (2.7-398.8)	28.7 * (0.8-250.1)	38.2 * (2.8-150.2)	30.4 * (2.0-165.3)	58.6 * (0.7-222.3)
<b>CD4<sup>+</sup>CD25<sup>high</sup> / μl</b>	0.5 (0.05-1.5)	0.9 (0.1-18.5)	1.3 * (0.2-14.8)	2.2 * (0.2-14.1)	2.9 * (0.1-30.4)	4.0 * (0.1-26.4)
<b>CD4<sup>+</sup>[%]</b>	3.6 (0.3-26.0)	6.5 (0.7-29.4)	7.3 (0.4-22.3)	6.6 (1.3-16.1)	8.0 (0.6-20.7)	10.2 (1.1-28.3)
<b>CD4<sup>+</sup>CD25<sup>high</sup> [%]</b>	5.7 (0.8-26.7)	3.8 (0.2-20.2)	6.0 (0.7-34.0)	7.6 (1.7-24.5)	8.8 (1.0-26.6)	7.6 (0.7-23.5)

D indicates days after PBSCT; \* p value < 0.05 compared to d30.

Table 3. Absolute and relative T cell reconstitution in acute/ chronic and chronic GvHD.

		CD4 <sup>+</sup> /μl	CD4 <sup>+</sup> CD25 <sup>high</sup> /μl	CD4 <sup>+</sup> [%]	CD4 <sup>+</sup> CD25 <sup>high</sup> [%]
d30	no GvHD	4.8 (1.8-59.8)	0.5 (0.4-1.2)	1.8 (0.7-22.8)	6.1 (2.0-26.7)
	a/cGvHD	10.3 (0.8-73.2)	0.2 * (0.05-0.64)	6.1 (0.3-25.2)	5.7 * (1.1-15.7)
	cGvHD	30.1 (6.4-65.3)	0.6 (0.1-1.4)	6.3 (2.3-26.0)	2.0 (0.8-11.1)
d60	no GvHD	25.9 (4.3-57.5)	1.0 (0.2-1.6)	4.7 (0.7-29.4)	2.3 (0.6-20.2)
	a/cGvHD	15.9 (2.7-103.7)	0.8 (0.1-1.6)	9.3 (1.8-24.8)	3.7 (0.9-22.0)
	cGvHD	28.7 (8.1-398.8)	1.4 (0.6-18.5)	9.4 (3.5-17.6)	7.2 (1.4-8.4)
d90	no GvHD	35.8 (3.1-112.2)	1.0 (0.3-4.6)	7.0 (3.3-16.8)	4.6 (1.9-9.6)
	a/cGvHD	19.2 (0.8-124.8)	1.1 (0.1-3.4)	6.8 (0.9-21.0)	6.4 (0.7-34.0)
	cGvHD	76.3 (17.8-250.1)	7.4 * (1.8-14.8)	11.7 (6.0-22.3)	9.8 * (4.6-18.9)
d120	no GvHD	37.6 (7.3-65.2)	1.4 (0.3-2.9)	6.5 (3.3-15.9)	3.9 (1.7-9.3)
	a/cGvHD	31.2 (2.8-150.2)	2.0 (0.2-6.2)	6.0 (1.3-16.1)	7.6 * (1.7-18.8)
	cGvHD	54.5 (11.4-115.6)	12.4 * (2.6-13.7)	10.0 * (6.6-15.6)	22.4 * (12.2-24.5)
d150	no GvHD	87.4 (8.0-113.2)	5.0 (0.3-7.1)	7.0 (3.7-19.0)	6.1 (3.4-9.1)
	a/cGvHD	22.8 (3.7-147.7)	2.1 * (0.1-6.5)	7.1 (0.6-20.7)	10.6 * (1.0-23.8)
	cGvHD	80.3 (17.2-165.3)	8.9 (0.5-30.4)	9.4 (4.0-16.6)	5.6 (3.0-26.6)
d180	no GvHD	103.4 (43.2-222.3)	5.3 (1.0-15.0)	9.8 (6.0-22.8)	6.7 (0.9-9.2)
	a/cGvHD	28.1 (0.7-187.6)	3.1 * (0.2-6.3)	7.6 (1.1-28.3)	8.3 (2.0-21.6)
	cGvHD	94 (18.8-147.2)	4.4 (0.9-26.4)	10.4 (2.5-19.2)	7.5 (1.6-23.5)

D indicates days after PBSCT; a/cGvHD acute/ chronic GvHD; cGvHD chronic GvHD; \* p value < 0.05 compared to no GvHD at the respective timepoint.

Table 4. T cell reconstitution in patients without clinical signs of GvHD with and without preemptive steroid therapy.

T cell reconstitution	d30		d60		d90		d120		d150		d180	
	no steroids	preemptive steroids	no steroids	preemptive steroids	no steroids	preemptive steroids	no steroids	preemptive steroids	no steroids	preemptive steroids	no steroids	preemptive steroids
CD4 <sup>+</sup> /μl	7.9 (6.1 – 59.9)	1.9 (1.8 – 3.5)	49.6 (13.8 – 57.5)	25.5 (4.3 – 26.4)	29.9 (10.3 – 112.2)	41.7 (3.1 – 59.2)	41.2 (30.7 – 57.2)	34 (7.3 – 65.2)	112.1 (71.5 – 113.2)	31.4 (8.0 – 103.4)	99.4 (70.8 – 222.32)	107.4 (43.2 – 143.2)
CD4 <sup>+</sup> CD25 <sup>high</sup> / μl	0.5 (0.5-1.2)	0.4 (0.2 – 0.5)	1.1 (0.3 – 1.4)	0.9 (0.2 – 1.6)	1.2 (0.5 – 4.6)	0.8 (0.3 – 3.6)	2.9 (0.7 – 3.0)	1.4 (0.3 – 1.4)	5.1 (5.0 – 6.1)	2.9 (0.3 – 7.1)	6.6 (3.8 – 15.0)	4.0 (1.0 – 11.5)
CD4 <sup>+</sup> [%]	3.4 (2.3 – 22.8)	1.0 (0.7 – 1.4)	6.2 (1.9 – 29.4)	3.1 (0.7 – 6.5)	7.7 (7.2 – 16.8)	4.2 (3.3 – 6.7)	7.9 (3.3 – 15.9)	5.1 (4.2 – 8.1)	6.3 (4.7 – 19.0)	7.7 (3.7 – 8.7)	10.6 (9.0 – 20.5)	7.4 (6.0 – 22.8)
CD4 <sup>+</sup> CD25 <sup>high</sup> [%]	4.0 (2.0 – 6.1)	24.5 * (6 – 26.7)	2.2 (2.0 – 2.3)	6.0 (0.6 – 20.2)	4.1 (4.1 – 5.1)	6.1 (1.9 – 9.6)	5.2 (1.7 – 9.3)	3.5 (2.2 – 4.2)	5.4 (4.5 – 7.0)	6.9 (3.4 – 9.1)	6.7 (5.3 – 6.8)	8.0 (0.9 – 9.2)

D indicates days after PBSCT; \* p value < 0.05 compared to patients that received no steroids at the respective timepoint.