CD52-negative T cells predict acute graft-versus-host disease after an alemtuzumab-based conditioning regimen

Pascal Woelfinger,1 Katharina Epp,1 Lukas Schafer,1 Diana Kriege,1 Matthias Theobald,1 Tobias Bopp2 and Eva-Maria Wagner-Drouet1
1Department of Hematology, Oncology and Pneumology, University Cancer Center Mainz (UCT), University Medical Center Mainz, and 2Institute for Immunology, University Medical Center of the Johannes Gutenberg-University Mainz, Mainz, Germany

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Correspondence: Pascal Woelfinger, Department of Hematology, Oncology and Pneumology, University Cancer Center Mainz (UCT), University Medical Center Mainz, 55131 Mainz, Langenbeckstraße 1, Building 605 and 302 Germany.
E-mail: pascal.woelfinger@unimedizin-mainz.de

Summary

Allogeneic haematopoietic stem cell transplantation (HSCT) after a reduced-intensity conditioning (RIC) regimen with fludarabine, melphalan and alemtuzumab is an effective therapy for haematological malignancies. Alemtuzumab, a monoclonal antibody against CD52, a glycosylphosphatidylinositol-anchor-bound surface protein on lymphocytes, depletes T cells to prevent graft-versus-host disease (GVHD). Despite this, acute and chronic GVHD (a/cGVHD) remain life-threatening complications after HSCT. The aim of the present study was to identify parameters to predict GVHD. In 69 patients after HSCT, T-cell subsets were functionally analysed. Reconstitution of CD52neg T cells and CD52neg regulatory T cells (Tregs) correlated with onset, severity and clinical course of aGVHD. Patients with aGVHD showed significantly lower levels of CD52pos T cells compared to patients with cGVHD or without GVHD (P < 0.001). Analysis of T-cell reconstitution revealed a percentage of <40% of CD52posCD4pos T cells or CD52pos Tregs at day +50 as a risk factor for the development of aGVHD. In contrast, CD52neg Tregs showed significant decreased levels of glycoprotein A repetitions predominant (GARP; P < 0.001), glucocorticoid-induced TNFR-related protein (GITR; P < 0.001), chemokine receptor (CXCR3; P = 0.023), C-C chemokine receptor type 5 (CCL5; P = 0.004), and increased levels of immunoglobulin-like transcript 3 (ILT3; P = 0.001), as well as a reduced suppressive capacity. We conclude that reconstitution of CD52neg T cells and CD52neg Tregs is a risk factor for development of aGVHD.

Keywords: GVHD, stem cell transplantation, T-cell depletion, T cells.

A reduced-intensity conditioning regimen (RIC) followed by allogeneic haematopoietic stem cell transplantation (HSCT) is an effective therapy to cure haematological malignancies. Acute and chronic graft-versus-host disease (a/cGVHD) are common and life-threatening complications after HSCT. One of the most effective ways to prevent GVHD is T-cell depletion (TCD) using alemtuzumab, a humanised monoclonal antibody against CD52.1 CD52 is a glycosylphosphatidylinositol (GPI)-anchor-bound protein on the surface of lymphocytes, monocytes, eosinophils and present on cells of the male reproductive tract.2 In alemtuzumab-treated patients >95% of CD3posCD4pos T cells and >80% CD3posCD8pos T cells were depleted.3

In 2019, Finazzi et al.4 showed that despite TCD with alemtuzumab the incidence of aGVHD Grades II–IV (Grades III–IV) was 34% (13%) and of cGVHD was 4%.

Many studies revealed that CD3posCD4pos and CD3posCD8pos T cells play a crucial role in the development of GVHD.5 Regulatory CD3posCD4posCD25posFoxP3pos T cells (Tregs) are responsible for induction and maintenance of self-tolerance and are required for prevention of GVHD.6–8 In 2010, we found that CD52neg T cells reconstitute after alemtuzumab-based TCD, donor CD3posCD4pos T cells convert mixed to full donor T-cell chimerism and replenish the CD52pos T-cell pool after alemtuzumab-based TCD.9 In the present study, we focussed on immune reconstitution of CD3posCD4pos, CD3posCD8pos T cells and Tregs after TCD with alemtuzumab in the context of GVHD and showed for the first time that reconstitution of CD52neg T cells is associated with development, severity and clinical course of aGVHD.

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Patients, materials, and methods

Patients and conditioning regimen

Five buffy coats, as healthy controls, and peripheral blood samples were collected from 69 patients who underwent HSCT at the University Medical Center of the Johannes Gutenberg University Mainz, Germany between 2010 and 2018. Patients were treated with RIC: fludarabine (30 mg/m² days −7 to −3), melphalan (140 mg/m² day 2) and alemtuzumab. All patients in our cohort received viral/anti-fungal/anti-microbial prophylaxis with aciclovir, posaconazole, cotrimoxazole/trimethoprim and ciprofloxac. GVHD prophylaxis contained cyclosporine A (day −1 to +50). Donor selection involved molecular typing for human leukocyte antigen (HLA)-A, -B, -C, -DRB1, and -DQB1 and were matched at least in eight of 10 HLA alleles.

The protocol was approved by the local ethics committees [837-185-00 (2551)] and national authorities. All patients gave written informed consent.

GVHD was staged by National Institute of Health criteria. Patients’ characteristics are summarised in Table I.

Chimerism analyses were performed by the Institute of Forensic Medicine in Mainz, Germany: DNA was extracted from bone marrow and analysed for chimerism by polymerase chain reaction (PCR) in a multiplex-PCR-System ‘PowerPlex ESX17’ (Promega, Walldorf, Germany). Detection limit for patient/donor cells ~5%.

Table I. Patient characteristics.

| Characteristic          | Total   | Acute GVHD | Chronic GVHD | No GVHD |
|-------------------------|---------|------------|--------------|---------|
| Total patients, n (%)   | 69      | 35 (50-7)  | 12 (17-3)    | 22 (31-8) |
| Age, years, mean (range)| 58 (27-75)| 58 (27-73) | 53 (36-68)  | 59 (39-75) |
| Sex, M:F, n             | 44:25   | 20:15      | 8:4          | 16:6    |
| Disease, n (%)          |         |            |              |         |
| AML                     | 24 (34-7)| 9 (25-7)   | 5 (41-6)     | 10 (45-5) |
| CML                     | 2 (2-8)  | 2 (5-7)    | 0 (0)        | 0 (0)   |
| MDS                     | 12 (17-3)| 9 (25-7)   | 1 (8-3)      | 2 (9-0)  |
| Lymphoma                | 14 (20-2)| 8 (22-8)   | 3 (25)       | 3 (13-6) |
| Myeloma                 | 14 (20-2)| 6 (17-1)   | 2 (16-6)     | 6 (27-2) |
| MPS                     | 2 (2-8)  | 0 (0)      | 1 (8-3)      | 1 (4-5)  |
| Others                  | 1 (1-4)  | 1 (2-8)    | 0 (0)        | 0 (0)   |
| Source of graft, n (%)  |         |            |              |         |
| MUD                     | 59 (85-5)| 32 (91-4)  | 11 (91-6)    | 16 (72-7) |
| Sibling                 | 10 (14-5)| 3 (8-6)    | 1 (8-3)      | 6 (27-2) |
| HLA matching, n (%)     |         |            |              |         |
| 10;10                   | 47 (68-1)| 22 (62-8)  | 7 (58-3)     | 18 (81-8) |
| 9;10                    | 19 (27-5)| 11 (31-4)  | 4 (33-3)     | 4 (18-1) |
| 8;10                    | 3 (4-3)  | 2 (5-7)    | 1 (8-3)      | 0 (0)   |
| CD3⁺ (×10⁶)/kg in the graft, mean, median ( range) | 243, 205, (7-879) | 280, 243, (7-879) | 155, 149, (104-251) | 215, 203, (121-325) |
| Full donor chimerism, n (%) | 61 (88)   | 31 (88)    | 10 (83)      | 20 (90)  |
| Virus reactivation, n (%) |         |            |              |         |
| EBV                     | 23 (33)  | 12 (34)    | 4 (33)       | 7 (31)   |
| CMV                     | 37 (53)  | 17 (48)    | 8 (66)       | 12 (54)  |
| Day of analysis post-HSCT, mean, median (range) | 243, 162, (40-2007) | 154, 120, (40-441) | 578, 352, (94-2007) | 204, 179 (48-480) |

Day of analysis post-HSCT provides the information on which day after HSCT blood draw for the comparison between the three GVHD groups was performed. M, male; F, female; HLA, human leucocyte antigen; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MPS, myeloproliferative syndrome; MUD, matched unrelated donor; EBV, Epstein–Barr virus; CMV, cytomegalovirus.
manufacturer’s protocol and stained with anti-Foxp3-FITC and anti-HELios-APC (eBioscience). For intracellular staining of cytotoxic T-lymphocyte antigen 4 (CTLA-4), Granzyme A and chemokine (C-C motif) receptor 5 (CCR5) a fixation/permeabilisation solution kit (BD Biosciences) was used according to the manufacturer’s protocol and stained with anti-CTLA4-APC, anti-Granzyme A-APC and anti-CCR5-APC (BioLegend, USA). Analyses were performed on a BD Biosciences fluorescence-activated cell sorting (FACS) Canto II Flow Cytometer and analyzed with BD FACSDiva Software (BD Biosciences).

Tregs isolation

PBMCs were labelled with anti-CD4-MicroBeads (MACS, Miltenyi) and isolated according to the manufacturer’s protocol followed by FACS with FACS Aria IIu and FACSDiva. Third party PBMCs were incubated with CD52pos or CD52neg Tregs of the same patient and proliferation was measured after 6 days. The cell suspension was incubated in AIMV medium (Gibco, Life Technologies, Carlsbad, CA, USA) and 10% human serum albumin and stimulated with recombinant human interleukin 2 (rhIL-2, 100 iu/ml) (Novartis, Basel, Switzerland) and OKT3 (30 ng/ml) (eBioscience) at day 0. Proliferation was evaluated by measuring carboxyfluorescein succinimidyl ester (CFSE) concentration performed with CellTrace® CFSE Cell Proliferation Kit (ThermoFisher, Waltham, MA, USA) according to the manufacturer’s protocol. PBMCs from six patients were incubated for 24 h with IL-2 and OKT3 and marker expression analysed by flow cytometry.

Statistics

Graph Pad Prism 5 software (Graphpad Software Inc., La Jolla, CA, USA) and the Statistical Package for the Social Sciences (SPSS®), version 23 (SPSS Inc., IBM Corp., Armonk, NY, USA) were used. For all analyses, a P < 0.05 was considered to be statistically significant. Non-parametric methods were used for group comparisons (Mann–Whitney test or Kruskal–Wallis test). Related group comparison for two groups was performed with Wilcoxon signed-rank test or Kruskal–Wallis test for more than two related groups.

Results

Reconstitution of leucocytes after alemtuzumab-based RIC HSCT

Absolute leucocyte count and lymphocyte counts did not differ significantly between patients with aGVHD, cGVHD or without GVHD (P = 0.23; P = 0.09).

Analysing relative counts of T cells, we found that patients with aGVHD had significantly higher amounts of CD3posCD4pos T cells (mean = 29.8%, median = 25.60%) compared to patients with cGVHD (mean = 17.15%, median = 12%; P = 0.033) or without GVHD (mean = 13.9%, median = 13.0%; P = 0.001). While, patients with cGVHD had significantly higher amounts of CD3posCD8pos T cells (mean = 30.11%, median = 28.3%) compared to patients with aGVHD (mean = 15.3%, median = 8.55%; P = 0.004).

CD52neg/GPIneg T cells reconstitute after alemtuzumab-based RIC HSCT

In healthy controls (n = 5) >98% of CD3posCD4pos, CD3posCD8pos T cells or Tregs expressed CD52. Patient-derived T-cell subsets [median (range) time of analysis after HSCT was 162 (40–2007) days; Table I] had a significantly lower frequency of CD52pos T cells: CD3posCD4pos T cells had the lowest CD52 expression (mean: 37.28%, median: 27.40%) followed by Tregs (mean: 50.30%, median: 44.50%) and CD3posCD8pos T cells (mean: 63.62%, median: 80.00%). CD52 expression differed statistically significantly between CD3posCD4pos T cells and CD3posCD8pos T cells (P < 0.0001), between CD3posCD4pos T cells and Tregs (P < 0.0001) and between CD3posCD8pos T cells and Tregs (P = 0.046).

CD52neg T cells lack GPI anchor consistently, indicating a loss of GPI-anchor expression on the lymphocyte surface, leading to missing surface expression of GPI-anchor bound molecules like CD52 (Fig 1).

Reconstitution of CD52neg T cells correlates with development of aGVHD

Reconstitution of CD52neg T cells differed significantly between patients with aGVHD, cGVHD or without GVHD: CD3posCD4pos T cells from patients with aGVHD expressed lower levels of CD52 (mean: 11.33%, median: 5.89%) compared to CD3posCD4pos T cells from patients with cGVHD (mean: 63.19%, median: 77.85%; P < 0.0001) and compared to CD3posCD4pos T cells of patients without GVHD (mean: 66.97%, median: 62.50%; P < 0.0001). There were no significant differences in CD52 expression of CD3posCD4pos T cells between patients with cGVHD and without GVHD (P = 0.65). Similar results were found within Tregs and CD3posCD8pos T cells: Tregs from patients with aGVHD also expressed lower levels of CD52 (mean: 16.31%, median: 5.56%) compared to Tregs from patients with cGVHD (mean: 75.47%, median: 91.00%; P < 0.0001) and compared to Tregs from patients without GVHD (mean: 91.30%, median: 94.50%; P < 0.0001). There were no significant differences in CD52 expression of Tregs between patients with cGVHD and without GVHD (P = 0.40). The CD3posCD8pos T cells of patients with aGVHD expressed the highest levels...
of CD52 within the T-cell subsets of patients with aGVHD (mean: 43-82%, median: 45-80%), but CD52 expression of CD3posCD8pos T cells was again significantly lower in comparison to patients with cGVHD (mean: 76-10%, median: 92-75%; P = 0.004) or without GVHD (mean: 88-60%, median: 90-65%; P < 0.001). Comparison of CD52 expression of CD3posCD8pos T cells between patients with cGVHD or without GVHD showed no significant differences (P = 0.59).

These results indicate that patients with a combination of high numbers of CD52posCD3posCD8pos T cells and low numbers of CD52pos Tregs and CD3posCD4pos T cells are at risk of aGVHD (Fig 2).

Longitudinal development of CD52 expression in T-cell subsets after alemtuzumab-based TCD
We analysed CD52 expression of T-cell subsets at different time-points in 12 patients who developed aGVHD, three patients with cGVHD and seven patients who never developed any GVHD. In patients who never developed any GVHD, CD52pos T cells reconstituted early after HSCT: >80% Tregs and CD3posCD8pos T cells expressed CD52 at day +50, but reconstitution of CD52posCD3posCD4pos T cells was later in comparison to CD52pos Tregs and CD52posCD3pos T cells of patients without GVHD, but faster in comparison to patients with aGVHD. Patients with aGVHD showed delayed reconstitution of CD52pos T-cell subsets in comparison to patients with cGVHD or no GVHD: none of the patients with aGVHD displayed >60% CD52posCD3posCD4pos T cells at day +350. Reconstitution of CD52pos T-cell subsets over time correlated with better clinical outcome. Three patients died of severe aGVHD. These patients showed very low CD52 expression in CD3posCD4pos T cells (<40%) and Tregs (<20%), but a high CD52 expression within the CD3posCD8pos T cells (up to 82%).

Referring to patients with cGVHD reconstitution of CD52pos T cells showed broad differences. One patient in the cGVHD group died of severe cGVHD of the skin and never reached >40% CD52 expression in Tregs and CD3posCD4pos T cells, but had high levels within CD3posCD8pos T cells (up to 100%). In summary, the results indicate a rate of <40% CD52posCD3posCD4pos T cells and CD52pos Tregs at day +50 as a predictive marker for the development of aGVHD (Fig 3).

Influence of Treg counts and functional Treg markers
In recent literature a low Treg count,13,14 altered Treg functions15–17 and changes of Treg-marker expression18 are described as risk factors for a development of GVHD. Therefore, we analysed the Treg count and expression of functional markers within each GVHD group, their expression on CD52pos and CD52neg Tregs, and performed functional analysis of CD52pos and CD52neg Tregs derived from the same patient.

In our cohort, PBMCs of patients with aGVHD contained less Tregs (% Tregs of CD3posCD4pos T cells mean: 3-63%, median: 1-91%) in comparison to patients with cGVHD (mean: 3-98%, median: 3-56%) and those without GVHD (mean: 5-4%, median: 3-61%), but with no significant differences (P = 0.25).

Cell surface receptor glycoprotein A repetitions predominant (GARP) is described as a marker of activated Tregs.19 GARP expression was higher on CD52pos Tregs (mean: 7-83%, median: 3-51%) compared to CD52neg Tregs (mean: 1-5%, median: 0%; P < 0.001). GARP expression on Tregs of patients with aGVHD (mean: 3-1%, median: 1-08%) was higher in comparison to patients with cGVHD (mean: 1-58%, median: 1-5%; P = 0.91), but significantly lower in comparison to patients without GVHD (mean: 4-32, median: 2-56).
Fig 2. CD52/GPI-anchor expression in different T-cell subsets predicts aGVHD. CD3posCD4pos T cells (A), CD3posCD8pos T cells (B) and Tregs (C) show significantly lower CD52/GPI-anchor expression in patients with aGVHD in comparison to patients with cGVHD or without GVHD. No significant differences in CD52/GPI expression were seen between the T-cell subsets of patients with GVHD or without GVHD. Significantly lower ratios of CD52posCD4pos/CD52posCD8pos T cells (D) and CD52pos Treg/CD52posCD8pos T cells (E) were found in patients with aGVHD. CD52pos Treg/CD52posCD4pos T-cell ratio (F) was not altered between the three groups. Dot plots with mean ± SEM are shown *P < 0.05, **P < 0.01, ***P < 0.001.

For CD39, CD44, CD62L, CD45RA, HLA-DR, Granzyme A and transcription factor HELIOS there were no significant differences between CD52pos Tregs and CD52neg Tregs or between the GVHD groups. The expression of CTLA4 was significantly higher in the Tregs of aGVHD patients (mean: 92-58%, median: 94-3%) compared to the Tregs of patients without GVHD (mean: 79-7%, median: 83-95%; P = 0.036) (Fig 4).

Functional analysis of CD52neg and CD52pos Tregs
To analyse potential differences of suppressive capacity between CD52pos or CD52neg Tregs, we isolated CD52pos and CD52neg Treg at one time-point from three different patients (patient 1: aGVHD, day 105 after HSCT; patient 29: aGVHD, day 126 after HSCT; patient 68: no GVHD, day 136 after HSCT) and compared their function in the above mentioned experiments. Analysing proliferation of CD3posCD8pos T cells after 6 days showed that CD52pos Tregs and CD52neg Tregs both were able to suppress effector T-cell proliferation, but with different efficiency: in all experiments CD52pos Tregs showed a stronger suppressive capacity than their CD52neg Treg counterparts. To gain more information about possible mechanisms of different suppressive capacities of CD52pos/neg Tregs we stimulated patients’ PBMCs (n = 6): GARP expression on patient Tregs was increased in both groups but to a greater extent in CD52pos Tregs (Fig 5).
Discussion

Reduced-intensity conditioning HSCT protocols using alemtuzumab, are feasible to treat older and pretreated patients with low tumour-related mortality; however, despite TCD GVHD occurs to some extent. Different schedules and levels of alemtuzumab can contribute to clinical effects and different T-cell reconstitution pattern. Patients treated with ‘proximal alemtuzumab’ (close to the time of graft infusion) developed more mixed chimerism and less aGVHD compared with patients treated with ‘distal alemtuzumab’ (more distant from the time of graft infusion) schedules, whereas ‘intermediate alemtuzumab’ (e.g. days −14 to −10) is described to reduce the incidence of mixed chimerism and is associated with a low incidence of aGVHD and decreases the need for additional haematopoietic cell products after HSCT. In a larger cohort of 101 patients treated in our clinic with the same ‘proximal alemtuzumab’ conditioning regimen, as in our present cohort, 46% of the patients developed aGVHD. In patients receiving a conditioning regimen without alemtuzumab, GPIneg/CD52neg T-cell populations are detected at the same low frequencies as in healthy individuals (<2%). In 2003 it was reported that alemtuzumab is detectable in patients’ serum up to 56 days after HSCT and that patients receiving alemtuzumab show a poor immune reconstitution, particularly with slow recovery of the CD3posCD4pos T-cell subset. In our present cohort of 69 patients after alemtuzumab-based RIC, reconstituting T cells lacked CD52 up to 2006 days after HSCT. This is consistent with previous publications by our group and others: Loeff et al. showed that lack of CD52 expression following alemtuzumab-based TCD results from loss of GPI-anchor expression caused by a highly polyclonal mutation frequency of the phosphatidylinositol glycan anchor biosynthesis Class A (PIGA) gene in T cells. Loeff et al. described the occurrence of very low frequencies of CD52neg T cells in the peripheral blood of healthy donors, suggesting that gaining mutations in the PIGA gene of T cells is a general
Fig 4. Treg marker expression. Significantly higher expression of GARP, GITR, CXCR3 and CCR5 were found in CD52pos/GPIpos Tregs. Converse results were observed relating to ILT3. CTLA4 was expressed significantly higher in the Tregs of patients with aGVHD. CD39, CD44, CD62L, CD45RA, HLA-DR, Granzyme A and transcription factor HELIOS showed no significant differences between the groups. Dot plots with mean ± SEM are shown *P < 0.05, **P < 0.01, ***P < 0.001.

Fig 5. Functional differences between CD52pos Tregs and CD52neg Tregs. Both, CD52pos Tregs and CD52neg Tregs were able to suppress effector T-cell (Teff) proliferation in MLR, but Teff incubated with CD52pos Tregs showed lower proliferation rates compared to Teff incubated with CD52neg Tregs (n = 3) (A). Steady state GARP expression in CD52pos Tregs and CD52neg Tregs (B) and GARP expression after stimulation for 24 h (C) showed higher GARP expression of CD52pos Tregs, an example is shown for one patient after stimulation (D). The expression of ILT3 strongly correlated with the expression of CD52/GPI anchor, here an example is shown in the CD3pos T cells of a patient after alemtuzumab-based TCD at day +146 (E). [Colour figure can be viewed at wileyonlinelibrary.com]
phenomenon, but indicating that treatment with alemtuzumab allows the preferential outgrowth of GPIpos/CD52pos T cells. Persistence of GPIpos/CD52pos T cells for years after HSCT may be caused by the long lifespan of memory T cells derived from the graft before they are replaced by new naïve GPIpos/CD52pos T cells from engrafted stem cells. We could show for the first time, that early and long-lasting reconstitution of GPIpos/CD52pos T cells significantly correlates with the onset and clinical course of aGVHD. Reconstitution of CD52pos T cells also correlates with mixed donor chimerism, possibly caused by higher selective pressure of CD52pos T cells after alemtuzumab treatment.9 Regarding function, some authors have reported that GPIpos/CD52pos CD3posCD4pos and CD3posCD8pos T cells in mice and men are functional,33,35,36 but these analyses solely focussed on effector T-cell functions, but exclude Tregs function.

Next to other modulators and triggers such as the micro-biome, drug therapy or tissue damage,37,38 CD3posCD4pos T cells and CD3posCD8pos T cells are described to be able to induce aGVHD.39,40 In the present study, we investigated our hypothesis that CD52pos T cells are functional and that CD52pos T cells lack function. Our present results confirm this hypothesis, as patients with aGVHD had higher levels of functional CD52posCD3posCD8pos T cells compared to the relative lower levels of functional CD52posCD3posCD4pos T cells in the aGVHD group (Fig 2). Data of longitudinal CD52pos T-cell reconstitution indicate that patients with aGVHD start with low levels of CD52pos cells before day +100 compared to patients that never developed any GVHD (Fig 3), pointing out that dysfunctional CD52pos T cells are a risk for the development of aGVHD generally. Our present data presume a value of <40% CD52posCD3posCD4pos T cells and CD52pos Tregs at day +50 as a predictive marker for the development of aGVHD. Particularly, a combination of high levels of CD52posCD3posCD8pos T cells and low levels of CD52posCD3posCD4pos T cells or CD52pos Tregs seem to identify patients at risk of developing aGVHD (Figs 2 and 3). Those patients are supposed to have functional CD52posCD3posCD8pos T cells as key drivers for the development of aGVHD and dysfunctional CD52negCD3posCD4pos T cells and CD52neg Tregs being unable to maintain immune homeostasis in these patients.

To date, functional data on GPIpos/CD52pos Tregs have been missing. Data from patients with multiple sclerosis treated with alemtuzumab (12 mg/day for 5 consecutive days, and again after 12 months for 3 days) also showed delayed CD3posCD4pos T-cell repopulation with expanding Tregs, but there was no monitoring of the CD52 expression.41 The in vitro studies of Havari et al.42 showed an increase in Treg frequency after alemtuzumab exposure of CD3posCD4pos T cells and adequate suppressive function dependent on both cell–cell contact and IL-2 consumption assuming an alemtuzumab-mediated effect promoting the long-term efficacy of alemtuzumab, but again without monitoring GPI-anchor or CD52 expression. CD52 is also described as a co-stimulatory molecule able to induce Tregs: in one study alemtuzumab treatment induced Tregs, which were able to suppress effector T cells in mixed lymphocyte reaction (MLR) but also without analysing CD52 expression.43 In the present study, we found for the first time that patient-derived GPIpos/CD52pos Tregs lack suppressive capacity in MLR and that they persist in high frequencies in patients with aGVHD. Testing functional Treg markers, GPIpos/CD52pos Tregs exhibit higher levels of GARP, GITR,46–48 CXCR3,10 and CCR5 confirming a more sufficient function and protection of aGVHD compared to their CD52pos counterparts. The role of ILT3 as a marker for reduced function in Tregs remains controversial. Ulges et al.44 described that ILT3pos Tregs negatively correlated with CK2pos Tregs, which enables Tregs to suppress T-helper cell type 2 responses in vivo, but not T-helper cell type 1 response. We found that ILT3 is almost only present on GPIpos/CD52pos T cells, suggesting it to be a GPI-anchored molecule as well (Fig 5E).

The role of CD52 in itself is not completely understood: Bandala-Sanchez et al.49 published data in which a suppressive population of CD52highCD4pos T cells inhibit CD52lowCD4pos T-cell activation through release of ‘soluble’ CD52 (sCD52) released by phospholipase C cleavage. sCD52 interacts with Siglec-10 on responder CD52lowCD4pos T cells and inhibits activation of responder cells by blocking lymphocyte-specific protein tyrosine kinase (Lck) and zeta chain of T cell receptor associated protein kinase 70 (Zap70) phosphorylation. These CD52highCD4pos T cells lack the molecular nomenclature of Tregs (CD25, Foxp3).50 Toh et al.51 also described immune modulation by CD52posCD3posCD4pos T cells by sCD52 or CD52-cross-linking as an option to suppress T-cell activity, suggesting CD52 as a suppressive molecule in CD3posCD4pos T cells.

Our present results give more insight into the complex interaction of T cells and drugs in the development and maintenance of GVHD, suggesting a prospective study on the clinical role of the reconstitution of CD52pos T-cell subsets with different schedules of alemtuzumab administration.

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Author contributions

Pascal Woelfinger planned the experiments, collected blood samples and performed the flow cytometry analysis and suppression assays, interpreted the data, and wrote the paper. Katharina Epp and Lukas Schaefer collected blood samples and performed the flow cytometry analysis and suppression assays. Diana Kriege collected blood samples and performed the flow cytometry analysis. Matthias Theobald carefully read the paper, and discussed the results. Tobias Bopp provided infrastructure for the suppression assays, read the paper, and
discussed the results. Eva-Maria Wagner-Drouet initiated and supervised the study, provided clinical data and wrote the paper. The manuscript contains parts of the doctoral thesis of Katharina Epp and Lukas Schaefer at the Department of Hematology, Oncology and Pneumology, University Cancer Center Mainz (UCT), University Medical Center Mainz.

Conflict of interest

The authors declare no conflicts of interest.

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