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T cell receptor repertoires after adoptive transfer of expanded allogeneic regulatory T cells

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Summary

Regulatory T cell (T_{reg}) therapy has been exploited in autoimmune disease, solid organ transplantation and in efforts to prevent or treat graft-*versus*-host disease (GVHD). However, our knowledge on the *in-vivo* persistence of transfused T_{reg} is limited. Whether T_{reg} transfusion leads to notable changes in the overall T_{reg} repertoire or whether longevity of T_{reg} in the periphery is restricted to certain clones is unknown. Here we use T cell receptor alpha chain sequencing (TCR-α-NGS) to monitor changes in the repertoire of T_{reg} upon polyclonal expansion and after subsequent adoptive transfer. We applied TCR-α-NGS to samples from two patients with chronic GVHD who received comparable doses of stem cell donor derived expanded T_{reg}. We found that *in-vitro* polyclonal expansion led to notable repertoire changes *in vitro* and that T_{reg} cell therapy altered the peripheral T_{reg} repertoire considerably towards that of the infused cell product, to different degrees, in each patient. Clonal changes in the peripheral blood were transient and correlated well with the clinical parameters. We suggest that T cell clonotype analyses using TCR sequencing should be considered as a means to monitor longevity and fate of adoptively transferred T cells.

Keywords: cell tracking, next generation sequencing, regulatory T cell therapy, T cell receptor repertoire

Introduction

Regulatory T cells (T_{reg}) have entered the clinic. Cell therapy with freshly isolated or expanded T_{reg} is applied in autoimmunity, transplantation and graft-*versus*-host disease (GVHD) [1–10]. Little is known about the stability and persistence of infused T_{reg} *in vivo*. Flow cytometry has been used to monitor T_{reg} quantitatively in the periphery post-transfer. Based on the expression of CD4, CD127, forkhead box protein 3 (FoxP3), and especially the high expression of CD25 on previously expanded T_{reg}, T_{reg} enumerations post-transfer could be tracked for 2 weeks in the case of third-party umbilical cord blood origin administered for GVHD prevention [9], and up to 3 months in the case of stem cell donor-derived T_{reg} for chronic GVHD (cGVHD) treatment [10]. Kinetics of transferred T_{reg} in the peripheral blood seem to vary greatly between individuals [1,6,10]. Recently, Bluestone *et al.* reported tracking of autologous expanded T_{reg} in type 1 diabetes patients by means of stable isotype labelling of T_{reg} by adding deuterium-labelled glucose during *in-vitro* expansion. Chromatography–mass cytometric analysis of isolated T_{reg} at different time-points post-cell therapy revealed 25% of peak labelling at 3 months and the detection of transferred T_{reg} in the periphery for 1 year [1]. However, whether longevity is independent of the specificity of T_{reg} or restricted to certain clones is unknown. We aimed to explore the feasibility of T cell receptor (TCR) next-generation sequencing (NGS) as a tool to measure T_{reg} clonality after expansion and *in-vivo* persistence after adoptive transfer, and as a means to track changes in the clonal repertoire of infused allogeneic T_{reg} with time. We chose T cell receptor (TCR)-α chain sequencing in this feasibility study, as this has been recently developed locally [11]. This is, to our knowledge, the first report exploiting T_{reg} TCR-α-NGS after adoptive transfer of T_{reg}.

Methods

Patient characteristics and T_{reg} therapy

TCR-α-NGS was performed in two patients who received adoptive T_{reg} therapy. Both patients suffered from treatment-refractory chronic GVHD as defined by National Institute of

Table 1. Patient characteristics.

	Type of disease	Conditioning/ GVHD prophylaxis	Stem cell source and characteristics (HLA match)	Donor gender and age	aGVHD	cGVHD organ manifestations at the time of T_{reg} infusion	Discontinued GVHD treatments prior to T_{reg} infusion	GVHD treatment at the time of T_{reg} infusion
Patient 1 male 54 years	B-CLL	Flu, Bu8/ CSA, MTX	BM allo unrelated (10/10)	Male, 39 years	Skin II°	Severe skin III° (progressive, maculopapular rash); oral cavity III° (progressive, ulcerations); eyes II° (stable, keratoconjunctivitis sicca)	Tacro, MMF, Alemtu, Rituxi, Dacli, Evero, ECP, MSC	Prednisolone (10 mg/d)
Patient 2 female 45 yrs	AML	Flu, 8 Gy TBI/CSA mono	PBSC allo related (10/10)	Female, 51 years	No	Severe skin III° (stable, ulcerations, sclerotic features); oral cavity II° (stable)	Tacro, MMF, ECP, MSC	Prednisolone (7.5 mg/d) Everolimus

Alemtu = alemtuzumab (3 × 10 mg subcutaneously per week); AML = acute myeloid leukaemia; B-CLL = B cell chronic lymphocytic leukaemia; BM = bone marrow; Bu = busulfan, Bu8 (8 mg/kg per os total); CSA = cyclosporin; cyclophosphamide (120 mg/kg total); Dacli = daclizumab (1 mg/kg); ECP = extracorporeal photopheresis; Evero = everolimus; Flu = fludarabine (30 mg/m^2/day for 5 days); MTX = methotrexate [graft-*versus*-host disease (GVHD) prophylaxis: cumulative dose 45 mg/m^2, days 1, 3, 6, 11 after haematopoietic stem cell (HCT); GVHD treatment: 5 mg/m^2 per week]; MMF = mycophenolate mofetil; MSC = mesenchymal stromal cells [average dose 1 × 10^6/kg body weight intravenously (i.v.)]; Rituxi = rituximab (100 mg i.v. per week); Tacro = tacrolimus; TBI = hyperfractionated total body irradiation (dose in Gy); HLA = human leucocyte antigen.

Health (NIH) criteria [12] after fully matched allogeneic haematopoietic stem cell (HSC) transplantation. The patients received T_{reg} infusions 40·5 months (patient 1) and 28 months (patient 2) after HSC transplantation (40 and 21 months after developing GVHD). Both patients showed full donor chimerism at the time of T_{reg} infusion. Details on original disease, graft characteristics, GVHD manifestation and discontinued GVHD medication are listed in Table 1. Patient 1 showed severe skin chronic GVHD (III°/progressive, maculopapular rash), affected oral cavity (III°/progressive, lichenoid buccal mucosal lesions/ulcerations) and eyes (II°/stable; keratoconjunctivitis sicca). Patient 2 reported severe chronic GVHD affecting the skin (III°/stable, ulcerations, sclerotic features) and the oral cavity (II°/stable). T_{reg} therapy and follow-up for both patients has been reported previously [10]. Briefly, T_{reg} were isolated from a leucapheresis product collected from the original haematopoietic stem cell donor by $CD8^+$ depletion and $CD25^{++}$ enrichment, expanded for 12 days with two rounds of αCD3αCD28 bead stimulation (Dynabeads Human T-Activator; Invitrogen, Carlsbad, CA, USA) and high-dose interleukin (IL)-2 (Proleukin S; Novartis Pharma, Basel, Switzerland) in the presence of rapamycin. Viability of the final cell product was 98% (patient 1) and 94% (patient 2), as determined by trypan blue staining. Cells were infused at a dosage of 3·7 × 10^6 T_{reg} cells/kg (patient 1) or 3·8 × 10^6 T_{reg} cells/kg (patient 2). Adoptive transfer of T_{reg} was conducted within a compassionate use programme. Immunomonitoring after T_{reg} therapy was performed after informed consent within a study protocol approved by the local ethics review committee (protocol no. EK 206082008).

Sample processing

Samples for TCR-α-NGS included unexpanded donor T_{reg} ($CD4^+CD25^{high}CD127^{low}$ T lymphocytes) and expanded donor T_{reg}, and recipient T_{reg}, $CD8^+$ T lymphocytes and $CD4^+CD25^+CD127^+CD45RO^+$ T lymphocytes obtained immediately prior to infusion and at several time-points after infusion (Table 3). Cells used for TCR-α-NGS were enriched magnetically and subsequently fluorescence activated cell sorter (FACS) purified after staining with CD4-peridinin chlorophyll (PerCP), clone SK3; CD25-phycoerythrin (PE), clone M-A251; CD45RO-allophycocyanin (APC), clone UCHL1 (all from BD Biosciences, San Jose, CA, USA) and CD127-eFluor450, clone eBioDDR5 (eBioscience, San Diego, CA, USA). The procedure is outlined schematically in Fig. 3b. Cells were lysed in RLT buffer (RNeasy MiniKit; Qiagen, Hilden, Germany) with 1% β-mercaptoethanol, snap-frozen and stored at −80°C.

Library preparation and NGS sequencing for TCR-α chain

RNA was isolated from the frozen cell pellets using the RNeasy MiniKit (Qiagen). First-strand cDNA was synthesized utilizing the template switching protocol for TCR-α,

and TCR-α amplified as described [11]. The final product was purified with the QIAquick polymerase chain reaction (PCR) purification kit (Qiagen). Barcoded libraries were pooled and 150 base pairs (bp) reads were generated using the Illumina HiSeq 2500 system. TCR CDR3 region sequences extraction and PCR error correction was carried out as described with MiTCR software (MiLaboratory, Moscow, Russia) [13,14]. Non-productive TCR sequences were filtered out, resulting in an average of 70% usable reads from the total reads obtained.

Statistical analysis

Analyses were conducted using R (2.15.0 2012-03-30; The R Foundation for Statistical Computing, Vienna, Austria) and Konstanz information miner (KNIME) [15]. Simpson's Diversity Index was determined as described [16], where an index of 0 is minimal and 1 is maximal diversity. In order to overcome the problem of differing sizes of different biological samples when comparing their Simpson's Diversity, we subsampled the same number of reads from each biological sample according to the smallest sample. This subsampling of reads was repeated 11 times, and the resulting Simpson Diversity indices were averaged. Pearson's correlation was calculated on proportions of clonotypes across time-points.

Results

Comparison of donor T_{reg} repertoire with preinfusion recipient T_{reg} repertoires

TCR-α reads within T_{reg} were compared in the SC) recipient and the HSC donor at the time of T_{reg} infusion, 40 months after bone marrow transplant (patient 1) and 28 months after peripheral blood HSC transplant (patient 2) (Fig. 1). There was no overlap in the 100 most frequent TCR-α clonotypes seen between the recipient and donor T_{reg} in patient 1. In contrast, in patient 2, 12 of the 100 most abundant clonotypes were shared between the recipient and donor. There was no obvious bias of these clonotypes with respect to their TCR-α variable (TRAV) and TCR-α joining (TRAJ) genes (data not shown).

TCR-α diversity is decreased after T_{reg} expansion

We were able to examine the diversity of the TCR-α repertoire in the pre-expansion and expanded T_{reg} in patient 2. Few cells were recovered for patient 1. *In-vitro* T_{reg} expansion of the T_{reg} for patient 2 was 18-fold, with a final purity of 91·8% $CD4^+CD25^{high}CD127^{low}FoxP3^+$ cells. We observed a similar gene usage pre- and post-expansion (Fig. 2a). Despite this, there were clear changes in the repertoire as assessed by comparing the frequency of clonotypes pre- and post-expansion (Fig. 2b, left panel). A large number of high-frequency clones from the isolated cell product were not detected in the expanded T_{reg} sample, whereas others that had frequencies of $< 0{\cdot}001\%$ in the isolated T_{reg} increased 100–1000-fold in their relative frequency in the expanded preparation. Of 987 clonotypes with read frequencies above 0·01% in the isolated cell product, 416 (42%) were not detectable (read frequencies $< 0{\cdot}0001\%$) in the expanded cell product. The 100 highest TCR-α clone frequencies were higher in the expanded T_{reg} preparations than in the isolated T_{reg} ($P < 0{\cdot}0001$) (Fig. 2b, right panel). Simpson's diversity indices of the isolated and expanded clonotypes after mathematical subsampling were 0·9996 in the pre-expanded T_{reg} and 0·9987 in the expanded T_{reg}.

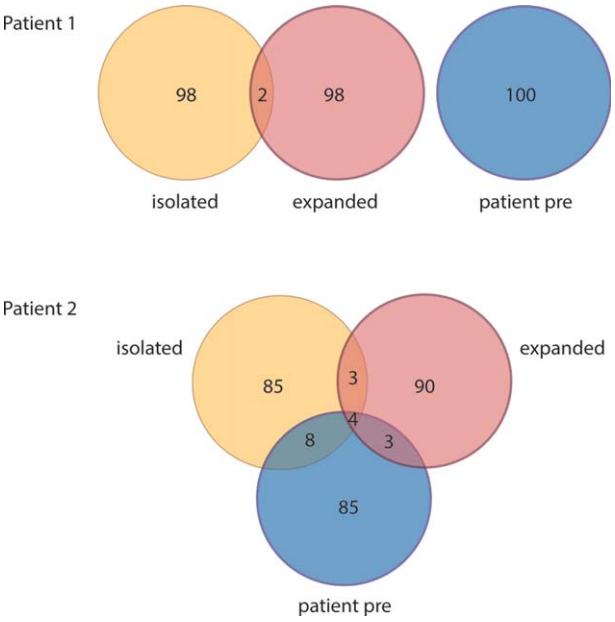

Fig. 1. Regulatory T cell (T_{reg}) clonotype overlap between stem cell donor and recipient. High-throughput T cell receptor (TCR)-α chain sequencing was used to define distinct T_{reg} clonotypes. Shown are Venn diagrams illustrating overlap in the 100 most frequent T_{reg} clonotypes found in donor-derived T_{reg} before (yellow), after *ex-vivo* expansion (red) and the recipient prior to T_{reg} infusion (blue). Upper diagram = patient 1, lower diagram = patient 2. Numbers illustrate numbers of distinct T_{reg} clonotypes.

T_{reg} TCR repertoire changes after T_{reg} infusion

We asked whether transferred T_{reg} can be detected in the periphery after infusion and whether the transfer leads to changes in the clonal distribution within the patient's T_{reg} cell pool. For patient 1, a 4.6-fold increase in the proportion of T_{reg} among the $CD4^+$ T cell pool was observed by flow cytometry 24 h post-infusion followed by a gradual decline from week 3 post-infusion (Fig. 3a, left graph). TCR-α-NGS sequencing of T_{reg} samples from this patient indicated a rapid change in the peripheral T_{reg} repertoire upon T_{reg} transfer, as shown by the lack of correlation comparing the repertoire pre- and 24 h post-transfusion (Fig. 4a, upper left plot and Fig. 4b, grey bars). Moreover, there was a marked

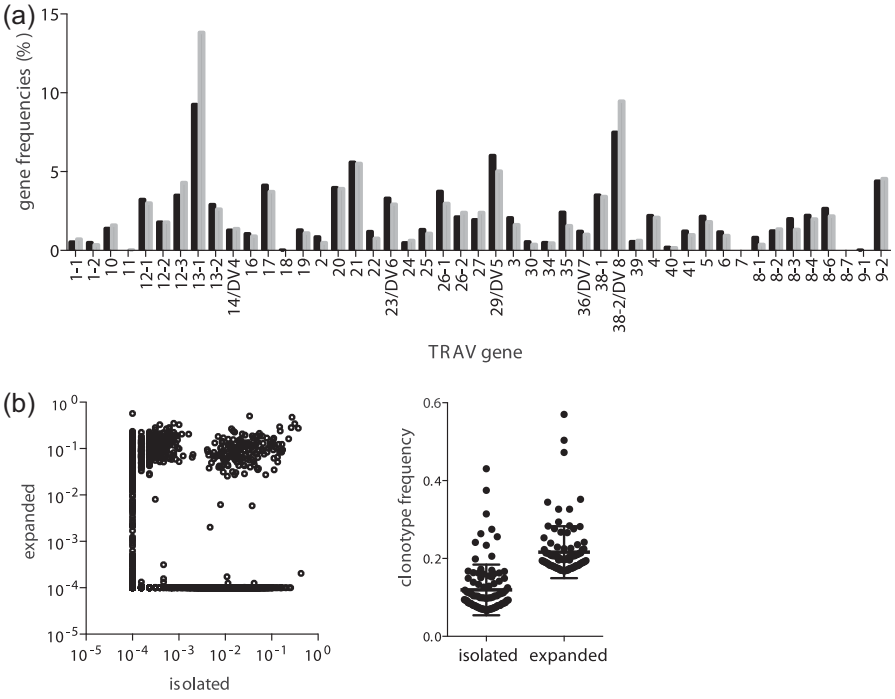

Fig. 2. T cell receptor (TCR) gene usage of regulatory T cell (T_{reg}) and clonotype frequencies before and after *in-vitro* polyclonal expansion. (a) High-throughput TCR-α chain sequencing was used to compare the gene usage of the T_{reg} pool before (black bars) and after *in-vitro* expansion (grey bars) for infusion into patient 2. Shown are the frequencies of the individual TCR-α variable (TRAV) genes. (b) Relative frequencies (in %) of all clones detected pre- or post-expansion were plotted in a correlation scatterplot (left). Clones that were not detected in one of the two samples (zero frequency) were set to 10^{-04}. The relative frequencies (in %) of the 100 most abundant clones are shown additionally in the right graph.

correlation between the repertoire of the expanded T_{reg} cell product and the patient's T_{reg} repertoire at 24 h post-transfer, suggesting that the increase in T_{reg} was a direct result of the infusion (Fig. 4a, lower left plot and Fig. 4b, black bars). The change in repertoires is also seen in the frequencies of the dominant TCR-α from the expanded T_{reg}, the preinfusion and the 6·5 weeks post-infusion repertoires (Fig. 4c, upper row). An increased correlation between the patient's T_{reg} repertoire and the expanded T_{reg} pool compared to patient's repertoire pre-T_{reg} transfer was observed for the first 3 weeks after infusion in this patient (Fig. 4a,b). At and after 6·5 weeks post-transfer the repertoire

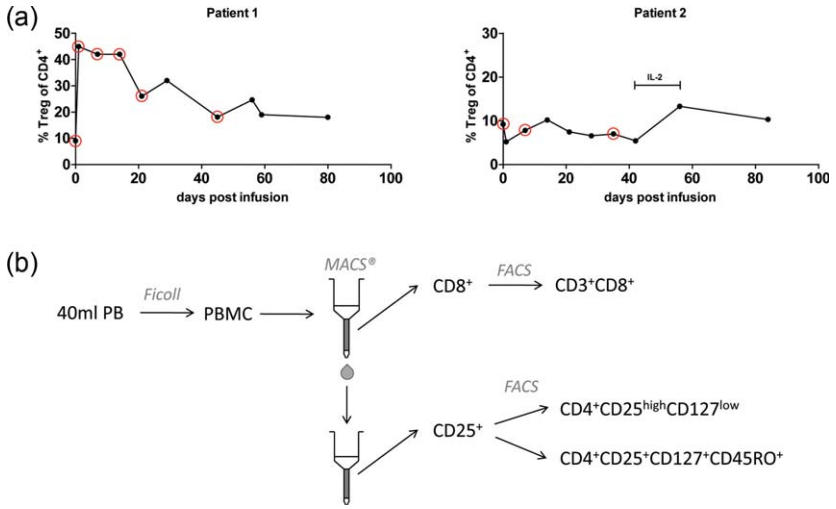

Fig. 3. Monitoring of peripheral regulatory T cell (T_{reg}) in patients before and after T_{reg} transfer over time. (a) Percentages of $CD4^+CD25^{high}CD127^{low}FOXP3^+$ T cells (T_{reg}) within the $CD4^+$ compartment were determined by flow cytometry. Circles indicate visits where additional blood samples were drawn for TCR-α sequencing studies. IL-2: patient 2 received low-dose IL-2 treatment later for the indicated time-frame. Left graph = patient 1, right graph = patient 2. (b) Layout of the cell isolation process for high-throughput TCR-α sequencing. Peripheral blood mononuclear cells (PBMC) were isolated by Ficoll density centrifugation from 40 to 50 ml of whole peripheral blood. $CD8^+$ cells were enriched by magnetic activated cell sorting (MACS) and subsequently sorted according to the expression of CD3 and CD8. The $CD8^-$ fraction was enriched for CD25 expression and sorted into T_{reg} ($CD4^+CD25^{high}CD127^{low}$) and $CD4^+$ memory T effector cells ($CD4^+CD25^+CD127^+CD45RO^+$).

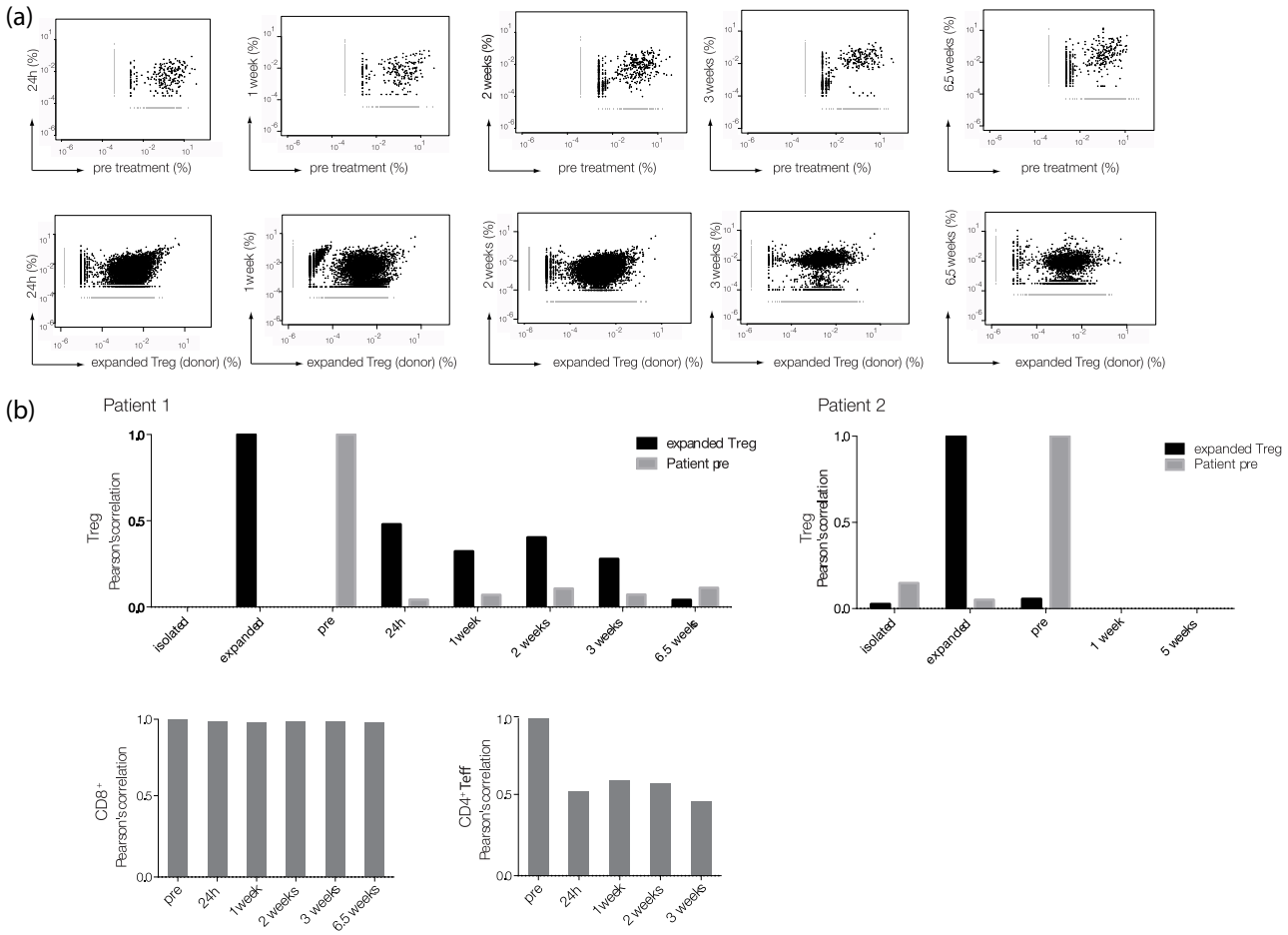

Fig. 4. Continued

correlation between the expanded cell pool and the patients' cell pool in the periphery decreased markedly. Most of the highest-frequency TCR-α in the sample 6·5 weeks post-infusion was represented in the preinfusion repertoire; few were found only in the expanded repertoire and some were found in both (Fig. 4c, upper panel). We did not detect a change in the $CD8^+$ T cell repertoire upon T_{reg} transfer within the monitored time-frame (Fig. 4b, lower left bar graph), and only minor changes in the repertoire of peripheral $CD4^+$ T effector memory cells (Fig. 4b, lower right graph). Interestingly, we observed a decrease in activation marker CD69-positive $CD4^+$ and $CD8^+$ T effector cells during the first 3 weeks post-T_{reg} transfer by flow cytometry in this patient [10]. This was followed by a transient clinical response affecting skin and oral mucosa (grades III–II) from weeks +3 to +8 post-therapy and a reduction of the corticosteroid dose (Table 2).

Despite infusion of a similar T_{reg} dose and a similar high viability of the cell product, the T_{reg} proportion among $CD4^+$ T cells in the peripheral blood of patient 2 did not increase after infusion as measured by flow cytometry (Fig. 3a, right graph). The T_{reg} TCR-α repertoires 1 and 5 weeks post-infusion showed little correlation with the preinfusion or the expanded T_{reg} repertoires (Fig. 4b, right panel). Patient 2 showed no clinical response but stable disease from week +1 to +4 post-T_{reg} therapy and GVHD progressed after week 5 (skin) (Table 2). Nevertheless, all the most frequent T_{reg} TCR-α found 5 weeks post-infusion were seen in the preinfusion or the expanded T_{reg} repertoires (Fig. 4c, bottom panels).

Discussion

Using NGS-TCR-α sequencing, we have demonstrated changes in T_{reg} repertoire following T_{reg} cell therapy. The changes were associated with clinical outcome, suggesting that monitoring TCR repertoires following adoptive T cell therapy may be considered as a measure of cell engraftment.

The study was exploratory to assess whether T_{reg} repertoires change after adoptive T_{reg} cell therapy. Despite being

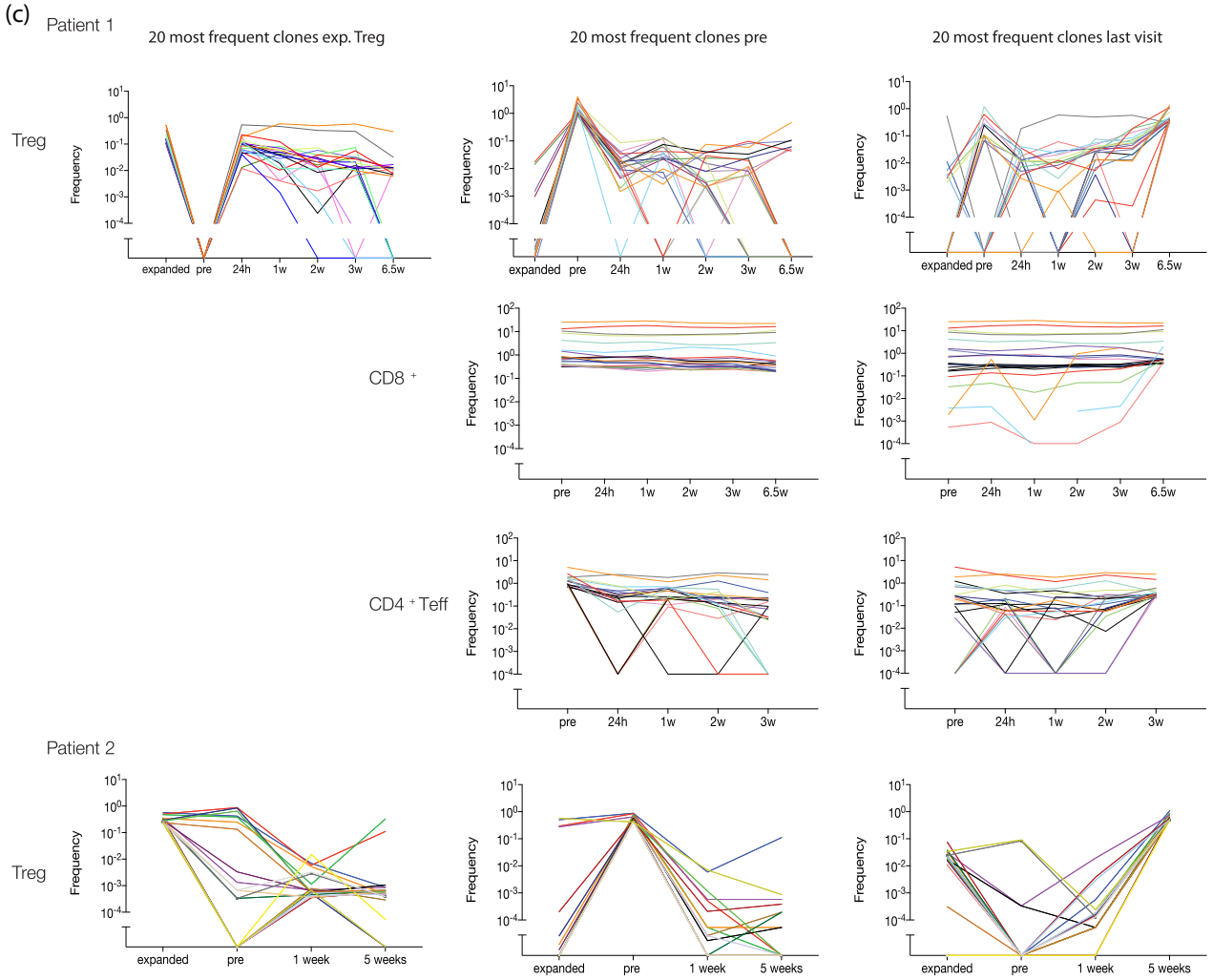

Fig. 4. Monitoring of regulatory T cell (T_{reg}) T cell receptor (TCR) repertoire changes after T_{reg} infusion. (a) Comparative frequency analysis of T_{reg} clonotypes using high-throughput TCR-α chain sequencing. Scatterplots were generated to compare relative abundance of T_{reg} TCR sequences at different time-points post-T_{reg} transfer to their relative frequencies (in %) pretreatment (first row) or to the relative frequencies (in %) of the sequences within the expanded T_{reg} pool (second row). Each dot represents a unique sequence. Sequences that were found only in one of the two compared samples are shown as light grey symbols. (b) Correlation analysis of the T_{reg} repertoires. Shown are Pearson's correlation coefficients as measures of the strength of the association between the TCR repertoire at different time-points post-T_{reg} transfer *versus* the TCR repertoire pre-T_{reg} therapy (grey bars) or *versus* the TCR repertoire of the expanded T_{reg} pool (black bars). Bar graph on left = patient 1, bar graph on the right = patient 2. (c) Tracking of the 20 most frequent T_{reg} clones of each sample over time. Every coloured line represents a particular clone. Plots on left: the 20 most abundant T_{reg} clones within the expanded T_{reg} pool followed throughout the indicated timepoints pre- and post-adoptive transfer for patient 1 (first row) and patient 2 (bottom row). Middle column: the 20 most frequent T_{reg} clones pre therapy and their frequencies over time (first row, patient 1; bottom row, patient 2). Second and third rows show the same analysis for $CD8^+$ T cells and $CD4^+$ effector memory T cells [$CD4^+$ T effector (T_{eff})] isolated from patient 1. Plots on right: the 20 most abundant T_{reg} clones at the last visit post-T_{reg} transfer and their frequencies before (first row: patient 1, last row: patient 2). The same analysis was conducted for $CD8^+$ (second row) and $CD4^+$ T_{eff} (third row) from patient 1.

small, and thus largely descriptive, it provides insight into the information that can be gained by extensive TCR repertoire analysis of specific cell types, and shows clearly that it can be a useful tool in adoptive T cell therapy. The following observations were of interest. First, follow-up in two patients demonstrated markedly different outcomes with respect to T_{reg} TCR repertoire changes. One patient (patient 2) had acquired a T_{reg} TCR repertoire 28 months after HSC transplant that overlapped substantially with that of the HSC donor with respect to frequent TCR-α clonotypes, and showed only minor changes in the peripheral blood T_{reg} TCR repertoire after T_{reg} cell therapy despite a high viability of the cell product at the time of infusion. In contrast, the T_{reg} TCR repertoire in the other patient (patient 1) was very different to that of the bone marrow donor, but changed dramatically to one resembling the

Table 2. Regulatory T cell (T_{reg}) graft characteristics and clinical response.

	T_{reg} purity after CliniMACS isolation (%$CD4^+CD25^{hi}$ $CD127^{lo}FoxP3^+$)	Duration of T_{reg} culture	T_{reg} fold expansion	T_{reg} purity after expansion (%$CD4^+CD25^{hi}$ $CD127^{lo}FoxP3^+$)	Total T_{reg} dose ($CD4^+CD25^{hi}$ $CD127^{lo}FoxP3^+$) ($\times 10^6$)	T_{reg} dose/kg ($CD4^+CD25^{hi}$ $CD127^{lo}FoxP3^+$) ($\times 10^6$/kg)	Time of T_{reg} infusion (months after HCT)	Clinical response during NGS follow-up
Patient 1 male 54 years	72·6	12 days	×4	84·1	4·00	3·71	40·5	Partial response week +3 to +8 (skin III°→II°; oral cavity III°→II°)
Patient 2 female 45 years	79·2	12 days	×18	91·8	4·72	3·76	28	Stable disease week +1 to +4; GVHD progression week +5 (skin)

GVHD = graft-*versus*-host disease; Fox P3 = forkhead box protein 3; NGS = next-generation sequencing; HCT = haematopoietic stem cell.

repertoire of the infused T_{reg} cells after adoptive T_{reg} cell therapy with a gradual return to a pre-adoptive T_{reg} cell therapy profile during a period of 1–2 months. The diverse outcomes in the two patients hold promise that monitoring TCR repertoires following adoptive T cell therapy may provide clinically meaningful information. Of note, the $CD8^+$ TCR repertoires did not alter after adoptive T_{reg} cell therapy, showing the specificity of the T_{reg} changes in the patients. The small initial change in the $CD4^+$ T effector repertoire is biased most probably by the small sample size of the pretransfer sample (Table 3). Donor characteristics differed between the two patients. Patient 1 received grafts from a 39-year-old unrelated matched donor and patient 2 from her 51-year-old sibling. Donor human leucocyte antigen (HLA) match and age have been shown to be associated with the risk of GVHD after allogeneic transplantation. We were also able to assess the T_{reg} repertoire after *in-vitro* expansion on one preparation. Several investigators aim currently at prolonged expansion cultures using modified expansion protocols of up to 35 days and to three rounds of restimulation driven by restricted starting material and/or to obtain higher T_{reg} doses [4,9,17]. A number of studies suggested polyclonality of the expanded cell pool using TCR Vβ repertoire analysis by flow cytometry [9,18]. Bluestone *et al.* reported recently the gene usage of bead-expanded T_{reg} by TCR-β sequencing before and after a 14-day expansion protocol. Looking at gene usage only, the cell product appeared polyclonal despite an average 500-fold expansion [1]. We had an 18-fold expansion and were able to confirm polyclonality and a stable gene usage by TCR sequencing. However, we revealed marked changes in the clonal repertoire accompanied by a considerable decrease in diversity after expansion. This contrasts the negligible observed repertoire changes in some reports [19,20]. This finding argues for further investigations by us and other sites aiming at and already reaching far higher numbers of *in-vitro* cell doublings before transfusion.

There are at least two limitations of all currently applied approaches to track T_{reg} after adoptive transfer. First, T_{reg} may undergo phenotypical changes including CD25 downregulation *in vivo*, as shown by Singh *et al.*, and thus might not have been isolated by FACS prior to further analysis [21]. However, the findings of Bluestone *et al.*, demonstrating that $CD4^+$ T cells other that T_{reg} did not show signs of deuterium labelling after sorting, suggest the plausibility of our approach [1]. As infused T_{reg} are $CD45RO^+$, the additional use of this marker for sorting post-infusion may improve the ability to track cells by TCR sequencing. The use of paired TCR-α and TCR-β sequencing using recent techniques [22] is also likely to improve tracking. Secondly, we are currently constrained to limit our analyses to peripheral blood. T_{reg} probably migrate to lymphoid tissue or sites of inflammation where they cannot be detected, and might thus be invisible to us, rather than cleared [23]. Their presence in the affected tissue might, at the same

Table 3. Sample cell numbers, obtained sequencing reads and clonotypes.

	Cell type/source	Sample/time-point	Cell number $\times 10^6$	Read number $\times 10^6$	Clonotype count
Patient 1	Donor T_{reg}	Isolated T_{reg}	0·040	0·25	6804
	Donor T_{reg}	Expanded T_{reg}	4·300	21·03	47 452
	Patient T_{reg}	Preinfusion	0·004	0·08	866
	Patient T_{reg}	24 h post	0·066	0·67	19 984
	Patient T_{reg}	1 week post	0·081	0·93	19 134
	Patient T_{reg}	2 weeks post	0·116	2·06	31 703
	Patient T_{reg}	3 weeks post	0·078	1·93	11 154
	Patient T_{reg}	6.5 weeks post	0·040	0·64	11 157
	Patient CD8	Preinfusion	0·470	5·98	7949
	Patient CD8	24 h post	0·510	6·77	9547
	Patient CD8	1 week post	0·900	8·42	17 370
	Patient CD8	2 weeks post	0·880	10·50	7401
	Patient CD8	3 weeks post	1·220	21·39	11 544
	Patient CD8	6.5 weeks post	1·400	20·52	15 489
	Patient CD4	Preinfusion	0·003	0·03	762
	Patient CD4	24 h post	0·005	0·05	1165
	Patient CD4	1 week post	0·008	0·07	5780
	Patient CD4	2 weeks post	0·007	0·12	2052
	Patient CD4	3 weeks post	0·010	0·23	4419
	Patient CD4*	6.5 weeks post	0·008	0·01	2103
Patient 2	Donor T_{reg}	Isolated T_{reg}	0·123	1·30	6810
	Donor T_{reg}	Expanded T_{reg}	2·560	12·80	3621
	Patient T_{reg}	Preinfusion	0·072	0·30	2957
	Patient T_{reg}	1 week post	0·270	6·23	30 152
	Patient T_{reg}	5 weeks post	0·199	1·04	1535

*Sample excluded from analysis for quality reasons. T_{reg} = regulatory T cells.

time, be of higher relevance for clinical benefit. Based on the similar product viability and dosage, we hypothesize that a retention in lymph nodes or a more rapid sequestration into peripheral tissue might explain the lack of evidence of infused T_{reg} in the peripheral blood of patient 2.

In conclusion, we found that TCR-α-NGS T_{reg} is a versatile method to track changes in the T_{reg} repertoire with time. Our results indicate that patients can partially adopt donor T_{reg} specificities after HSC transplantation, and that adoptive T_{reg} cell therapy can lead to transient clonal changes within the circulating peripheral T_{reg} repertoire. The degree of these repertoire changes can differ substantially between individuals. Some of the transferred T_{reg} clones appeared to reside longer in the periphery than others, and overall clonal changes are of transient nature. Thus, we advocate the use of TCR repertoire analyses, together with analyses such as the use of deuterium labelling of cells in patients undergoing adoptive T cell therapies.

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Disclosures

The authors declare no commercial, proprietary or financial interests in the products or companies described in this paper.

Author contributions

A. T. planned and supervised T_{reg} isolation and expansion, planned and performed immunomonitoring and FACS sorting and drafted the manuscript; C. W. performed MACS and FACS sorting, M. K. performed data analysis, A. P. performed sequencing data preprocessing, S. T. compiled clinical data, U. O. supervised immunomonitoring for patient 2, A. D. performed sequencing, M. B. initiated and supervised T_{reg} cell therapy and critically read the manuscript, E. B. initiated, planned and supervised the study and contributed to manuscript writing; A. E. planned and supervised the study, performed T cell receptor library preparation, data analysis and contributed to manuscript writing.

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