Tuesday, March 28th

16:30-18:00

Oral 13: MRD, chimerism and immune reconstitution

A Rapid and Sensitive Molecular Tool for the Early Diagnosis of HLA Loss Relapses after Partially-Incompatible Allogeneic HSCT

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Abstract

Introduction. Genomic loss of the mismatched HLA haplotype represents a frequent mechanism of leukemia immune evasion and relapse after partially-incompatible allogeneic HSCT, accounting for up to one third of relapses after haploidentical HSCT and a yet unknown proportion of relapses after unrelated donor HSCT. Detection of HLA loss variants has relevant clinical implications, since infusion of lymphocyte from the donor is expectedly ineffectual against these forms of relapse. Thus, sensitive, reliable and easy to perform assays are needed to render this diagnosis possible to all centers performing partially HLA-mismatched HSCT. Material and methods. Here we designed an innovative methodology to detect HLA loss relapses, based on the combination of quantitative PCR (qPCR)-based chimerism to detect host markers ("outside HLA" markers) with ad hoc designed qPCR reactions targeting the most frequent HLA allele groups ("inside HLA" markers). Concordance between the inside and outside HLA markers identifies classical (non-HLA loss) relapses, whereas presence of host-specific outside HLA markers with concomitant negativity of the patient-specific HLA identifies HLA loss relapses. For "outside HLA" markers, a commercial qPCR chimerism assay targeting 29 in/del polymorphisms was used (KMRtype and KMRtrack assays, GenDx, Utrecht). For "inside HLA" markers we designed qPCR reactions specific for the most frequent HLA-A, -C and -DPB1 allele groups, targeting both locus-specific and allele group-specific polymorphisms. We tested the specificity, efficiency and sensitivity of each reaction using HLA-typed reference DNAs, serially diluted in water and in artificial chimeric mixtures, and validated the utility of the reactions with samples from patients who experienced classical (n=4) or HLA loss (n=5) relapses. Results. We designed and validated a total of 10 "inside HLA" reactions, capable to provide at least one informative HLA marker to over two thirds of a representative series of 165 consecutive patients who received haploidentical HSCT at our Institute. Each reaction was tested against a panel of HLA-typed cell lines (n>20) to confirm specificity and absence of crossreactivity. All the reactions displayed over 80% efficiency, with superimposable performance in water and in target-negative DNA (R=0.99, p<0.0004). Accuracy and precision in chimerism determination resulted very high for all the reactions, with almost perfect concordance between the expected and experimentally-determined quantification (R= 0.99, p<0.0001). Maximal reproducible sensitivity (defined as the lowest dilution in which all replicates are positive within a 1.5 cycle threshold range) was at least 0.2% for all the reactions. The clinical utility of the newly developed assays was confirmed analyzing 9 cases of relapse after partially HLA-mismatched HSCT. In all cases we detected host-specific chimerism using outside HLA markers and, as expected, HLA markers resulted positive in case of classical relapses (4/4) and negative for HLA loss relapses (5/5). Conclusion. We developed a highly sensitive, reliable, and easy-to-implement molecular tool to unequivocally discriminate between classical and HLA loss relapses, facilitating further retrospective and prospective studies and allowing the implementation of this differential diagnosis in the therapeutic algorithm for post-transplantation relapses.
Disclosure of conflict of interest

E.B. and W.M. are employees of GenDx; K.F. and L.V. received research funding from GenDx
Immune Monitoring in Allogeneic Hematopoietic Stem Cell Transplant Recipients: a Survey from the EBMT-Cellular Therapy & Immunobiology Working Party

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Abstract

Background: Post transplant immune reconstitution plays a major role in determining the outcome of allogeneic hematopoietic stem cell transplantation (allo-HSCT), and is currently monitored with different techniques in different Centers, with the aim of identifying clinically relevant immunological biomarkers. However, it is unclear which and how many of these tests are currently performed on a routine basis, and which ones have the potential to guide patient care after allo-HSCT. Methods: The EBMT Cellular Therapy & Immunobiology Working Party (CTIWP) conducted a survey to identify current policies to monitor immune reconstitution in patients undergoing allo-HSCT and possibly reach a general consensus. This study followed the EBMT study guidelines. All EBMT Centers were invited to participate. Each participating Center received a questionnaire on the availability of specific immunomonitoring assays, specifying the use in clinical practice and/or within investigational trials. Results: Policies for post-transplant immunomonitoring have been reported by 56 participating EBMT Centers active in 19 Countries and performing allo-HSCT from HLA identical related (56 centers), matched unrelated (54), haploidentical (55), unrelated cord blood (50). Complete blood counts and immunoglobulins are routinely tested for patients’ care by all centers. Relative proportions of T cell subsets are currently tested by flow-cytometry as “standard of care” or “investigational” by 82% and 36% of centers respectively. B cell and NK cell counts are quantified routinely by 43% and 23% of Centers, and investigationally by 54% and 55% of Centers. The availability of molecular tests (STR, qPCR, Fish) to measure post-transplant engraftment are reported by all Centers, except three, as a standard of care measure. T cell receptor–expressing circles (TRECs) and/or K-deleting recombination excision circles (KRECs) are quantified within selected clinical trials by 36% of Centers. Interestingly, 66% of Centers evaluate, mostly as an investigational measure, antigen specific T cell responses by: proliferation assays (50%), interferon-gamma enzyme-linked immunospot-Elispot (45%), intracellular cytokine staining (48%) and tetramer/dextramer staining (34%). Most of these Centers test responses to Cytomegalovirus and Epstein Barr Virus, and 14 Centers use at least one of these assays on a routine basis. Most of the participating Centers (68%) commonly test antigen-specific antibodies, mainly as responses to vaccines, and not routinely. T-cell receptors (TCR) and B-cell receptors (BCR) repertoires are measured by spectratyping in 20 out of 56 Centers (7 as clinical practice and 13 in selected trials), or, in selected trials, by next generation sequencing (in 18 out of 56 the participating Centers). Conclusions: Results of this survey indicate that country- and center expertise are associated with heterogeneous and distinct protocols, and underline the clinical need to harmonize methods and to provide
practical recommendations for monitoring post-transplant immune reconstitution, both for routine purposes and investigational studies. Adequate reporting and connection between individual Centers exploiting these data will foster collaborative and comparative research studies, with the ultimate goals of improving patient care and refining our understanding of the immunological correlates to clinical outcome.

**Disclosure of conflict of interest**

The authors declare no competing financial interests.
Multiple Inhibitory Receptors Are Expressed on Central Memory and Memory Stem T Cells Infiltrating the Bone Marrow of AML Patients Relapsing After Allo-HSCT

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Abstract

Background: In patients with acute myeloid leukemia (AML), relapse is the major cause of death after allogeneic HSCT. Patients and Methods: To investigate whether T-cell dysfunction is associated with post-transplant relapse, we longitudinally analyzed bone marrow (BM) and peripheral blood (PB) samples of 32 AML patients receiving HSCT from HLA-matched (HLAid, 20 pts) or HLA-haploidentical (haplo, 12 pts) donors. BM and PB were collected 60 days after HSCT and at relapse (median 251 days; 16 pts) or at 1 year in case of complete remission (CR; 16 pts). Samples from 10 healthy donors (HD) were used as controls. The expression of inhibitory receptors (IRs) on T-cell subsets was evaluated by multi-parametric flow cytometry. Results were analyzed with both the FlowJo software and the BH-SNE algorithm, an unbiased computational method. To evaluate T-cell effector functions, the CD107a degranulation assay was performed and the production of cytokines was measured by intracellular staining. The ability of BM T cells to kill AML blasts after in-vitro rapid expansion protocol (REP) was tested in co-culture experiments. Results: We investigated the expression of PD-1, CTLA-4, KLRG1, LAG-3, 2B4 and Tim-3 as T-cell exhaustion markers. After haplo-HSCT, multiple IRs were significantly upregulated in BM and PB T cells at all time-points, compared to HD and independently from the clinical outcome. Conversely, after HLAid-HSCT, patients who relapsed, displayed a higher frequency of BM infiltrating T cells expressing PD-1, CTLA-4 and Tim-3 than CR pts (p<0.05) and HD (p<0.01). Results were confirmed by using the BH-SNE algorithm. We then investigated the profile of each memory T-cell subset in our cohort. In the BM of HD and CR-patients the expression of IRs was confined to late differentiated T cells. Differently, at relapse, PD-1, 2B4 and Tim-3 were also upregulated in BM infiltrating central memory (p<0.01) and memory stem T cells (p<0.05). To verify whether the phenotypic profile of T-cell exhaustion at relapse associates with functional impairment, we evaluated T-cell effector functions upon polyclonal stimulation. We observed a lower degranulation ability of both CD62L+ early differentiated (p<0.01) and CD62L- late differentiated (p<0.05) CD8 cells at relapse when compared to CR and HD. In 4 patients, we isolated and expanded by REP T cells expressing one or more IRs (IR+) or no IR (IR-). Expansion rates were high in both IR+ and IR- cells (mean fold increase 490 and 992, respectively at day 21). The degranulation ability measured ex vivo in those patients (mean 9.2%) was dramatically increased upon REP expansion (80.0% and 79.9% for IR+ and IR-, respectively; p<0.001). Similarly, the frequency of IFNg/TNFa producing CD8 cells increased in IR+ and IR- cells upon REP (p<0.05), indicating that the T-cell dysfunction observed at relapse can be efficiently reversed. We next co-cultured IR+ and IR- cells with autologous leukemias. Strikingly, IR+ cells showed a greater ability to kill AML blasts (elimination index, EI=68%) compared to IR- cells (EI=40%; p<0.05) at low effector to target ratio, suggesting that IR+ cells are enriched in leukemia specificities. Conclusions: After HSCT, the signature of exhausted T-cell in relapsing pts includes PD-1, CTLA-4, 2B4 and Tim-3. The IRs expression on early differentiated T cells at relapse suggests a wide, though reversible, immunological dysfunction mediated by relapsing AML blasts.
Disclosure of conflict of interest

None
Dynamics of expression of Programmed cell death protein-1 (PD-1) on T cells after allogeneic hematopoietic stem cell transplantation

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Abstract

Introduction: Blockade of the programmed-death 1 (PD-1) immune checkpoint represents a promising strategy to enhance anti-tumoral immune responses after allogeneic hematopoietic stem cell transplantation (allo-HSCT)1,2. However, observational studies suggest that PD-1 blockade can be complicated by the development of severe graft-versus-host disease (GvHD)2,3. A better knowledge of the dynamics of PD-1 expression by T cells after allo-HSCT is necessary in order to optimize PD-1 targeting therapies and limit toxicities. 2. Patients and Methods: We analyzed by flow cytometry 124 freshly drawn blood samples isolated from 98 allo-HSCT recipients. Twenty-three healthy blood donors served as controls (HC). 3. Results: We observed a strong increase in PD-1 expression at the surface of CD4 and CD8 T cells isolated from allo-HSCT recipients compared with HC (Fig 1A). Importantly, we observed the significant increase of PD-1 expressing cells in all CD4 and CD8 T cell subpopulations studied including naïve, central memory (CM), effector memory (EM) and terminal EM CD45RA+ (TEMRA) cells (Fig. 1B). We observed an inverse correlation between the time since allo-HSCT and PD-1 expression at T cell surface (Fig. 1C). PD-1 expressing cells were higher than normal already at one month after allo-HSCT (Fig. 1D). Thereafter, the proportions of CD4 PD-1+ T cells remained higher than in HC up to more than 5 years after HSCT, while the PD-1 expression on CD8 T cells started to normalize from 1 year after transplantation on (Fig. 1D). The stem cell source (BM vs PBSC), conditioning regimen (RIC vs MAC), use of total body irradiation and disease status at HSCT did not impact PD-1 expression. We observed higher proportions of PD-1+ CD4 but not of PD-1+ CD8 T cells in patients having received in vivo and/or ex vivo T-cell depletion (TCD) compared with patients receiving T cell replete grafts (p=0.0269). CD8 T cells from patients receiving grafts from haploidentical donors expressed higher proportions of PD-1+ cells than CD8 T cells from patients receiving grafts from matched related (p=0.0492) or unrelated donors (p=0.0049). No association was found between PD-1 expression on T cells and post-transplant complications, including acute or chronic GvHD, disease relapse and CMV reactivation. 4. Conclusion: We report here a rapid and long lasting increase of PD-1 expression by CD4 and CD8 T cells after allo-HSCT. Several factors, including TCD and transplantation from haploidentical donors, are associated with a further increase in PD-1 expression on T cells. These results will help harnessing the potential of PD-1 blockade after allo-HSCT. 5. References: 1 Villasboas et al., Oncotarget (2016) 2 Haverkos et al., Blood 128.22 (2016): 1163 3 Singh, A. K., et al. Bone marrow transplantation (2016) 6. Image: (A) Expression of PD-1 on T cells after allo-HSCT. (B) Expression of PD-1 on T cell subsets (naïve: CD45RA+ CCR7+; CM: CD45RA- CCR7+; EM: CD45RA- CCR7- and TEMRA: CD45RA- CCR7-). (C) Relationship between PD-1 expression and days after HSCT. Median percentage (dashed line) and interquartile range (gray area) of PD-1 expression from HC. (D) Proportions of PD-1 expressing cells at different time-points after HSCT.
Disclosure of conflict of interest

No conflicts of interest
Relapse of Myeloid Malignancies after Allogeneic Stem Cell Transplantation – a single Center Analysis

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Abstract

Introduction: Allogeneic stem cell transplantation (allo-SCT) can cure a considerable number of patients with high-risk myeloid malignancies, but still relapse remains the major cause of treatment failure. We retrospectively studied relapse kinetics, treatment and outcome of AML and MDS after allo-SCT at our center during a 13 year period. Patients and Methods: A total of 447 pts (median age 52 years, range 17-72, 55% male) received their first allo-SCT from a related (31%), unrelated (67%) or combined donors (2%) for myeloid malignancies at the University Hospital Dusseldorf between 2002 and 2015. Diagnoses were: 217 de novo AML (AML, 49%), 103 MDS (23 %), 53 MPN (12%, 32 CML, 21 OMF) and 74 sAML (16%, 69 evolving from MDS and 4 from MPN). Median follow up was 23.2 months (range 0.02-167.2). Results: Median survival after allo-SCT of the whole cohort was 51.3 months (range 0.02-167.2). A total of 160 patients (35.8%) relapsed after a median of 4.5 months (range 0.43-110.4). The majority (113, 70%) were hematologic relapses (HR), 33 pts (21%) had molecular or cytogenetic relapses (MR) and 14 patients had extramedullary relapses (9%, XR, 8 isolated and 6 combined with HR). The great majority of relapses (115, 73%) occurred within the first 3 years (1st year: 73%, 1st + 2nd year: 88%, 1st + 2nd + 3rd year 96%). Only 7 pts relapsed beyond the 3rd year. Median time to relapse differed by diagnosis (AML 3.7 months, range 0.43 – 88.6, MDS 14.7 months, range 0.9-110 ms; MPN 5.6 months, range 0.6-90 ms, sAML 3.5 months, range 0.9-61.5, AML vs MDS p=0.002, MDS vs sAML p=0.005, MPN vs AML p=0.045, MPN vs sAML p=n.s.). Median survival time after relapse was 8.4 months and 2-year survival was 31.3 %. A total of 119 (74.4%) patients died a median of 4.6 month after relapse (range 0.2-105) and 41 (25.6%) are alive after treatment for relapse (9 with disease and 32 in CR). Following relapse, the majority of pts (n=143, 89%) received at least one salvage therapy, while 9 pts received BSC only (8 pts no information).

Primary salvage therapies were hypomethylating agents (HMA, n=101 azacytidine, n=1 decitabine), intensive chemotherapy (n=10), sorafenib (n=11) and radiotherapy (n=7). DLI were applied in 85 pts and 2nd allo-SCT was performed in 19 pts. Type of treatment changed over time with increasing use of HMA (n=39 before 2009 vs n=63 after 2009, p<0.001) and treatment results improved (median OS 4.5 months before 2009 vs 10.5 months after 2009, Breslow p=0.005, 2 year OS 27% before vs 36% after 2009). Patients who were treated for MR had a better prognosis than patients with HR (OS 26.3 months vs 4.8 months, p<0.001) as had patients with MDS vs AML (OS 89.3 months vs. 5.6 months, p<0.001). Of 36 patients who survived in remission > 2 years 23 (63.9%) had received HMA and DLI, 6 (23.1%) had received a second transplant, 2 DLI only, 4 tyrosinekinase-inhibitors, 2 interferone alpha and DLI and 1 intensive chemotherapy. Conclusion: A substantial number of patients who relapse with myeloid malignancies after allo-SCT can re-achieve remission and long term survival, especially with HMA+DLI or 2nd allo-SCT. Relapse of MDS carries a better prognosis than AML. Techniques to further improve the detection of MRD are urgently needed because early treatment of molecular relapse results in significantly better survival.

Disclosure of conflict of interest

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Unmanipulated haploidentical donor transplantation is superior to matched sibling donor transplantation in eradicating pre-transplantation minimal residual disease as determined by multiparameter flow cytometry

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Abstract

INTRODUCTION This study compared the effects of pre-transplantation minimal residual disease (pre-MRD) on outcomes in AML patients who underwent human leukocyte antigen-matched sibling donor transplantation (MSDT) or who received unmanipulated haploidentical allografts. PATIENTS AND METHODS We compared outcomes in 340 AML patients who received MSDT (n=82) versus unmanipulated haploidentical allografts (n=258). MRD was determined using multiparameter flow cytometry. RESULTS Patients who received allografts from haploidentical donors and MSDT had similar 2-year probabilities of leukemia-free survival (LFS) (P=0.703) and overall survival (OS) (P=0.142). Patients with negative pre-MRD had a lower incidence of relapse than those with positive pre-MRD in MSDT settings (7% vs. 38%, P<0.001), but relapse was comparable in haploidentical allograft settings for patients with negative pre-MRD (8%) versus positive pre-MRD (13%, P=0.167). Of the patients with positive pre-MRD (n=76), those who underwent MSDT had a higher incidence of relapse (38%) than those receiving haploidentical allografts (13%; P=0.017) plus lower probabilities of LFS (54% vs. 80% P=0.007) and OS (64% vs. 83%, P=0.062). Multivariate analysis showed that for pre-MRD-positive AML patients, haploidentical allograft was associated with a low incidence of relapse (HR, 0.131; 95% CI, 0.037–0.467; P=0.002) and with better LFS (HR, 0.221; 95% CI, 0.085–0.574; P=0.002) and OS (HR, 0.325; 95% CI, 0.111–0.952; P=0.040). CONCLUSIONS Pre-MRD had no negative effects on outcomes after haploidentical transplantation. For pre-MRD-positive AML patients, haploidentical allograft was associated with lower incidence of relapse and better survival compared with MSDT. This suggests it is better to eradicate pre-MRD and has the stronger graft-versus-leukemia effects.

References


Figure 1. Relationship between pre-stem cell transplantation minimal residual disease (pre-SCT MRD), as determined by multiparameter flow cytometry, and transplant outcomes for pre-MRD positive patients with acute myeloid leukemia (n=76). Kaplan-Meier estimates of (A) cumulative incidence of relapse and (B) leukemia-free survival.
Disclosure of conflict of interest

The authors declare no conflict of interest
Molecular monitoring of acute myeloid leukemia using targeted ultra-deep sequencing

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Abstract

Introduction Molecular monitoring based on assessment of minimal residual disease (MRD) is becoming increasingly more important after allogeneic stem cell transplantation (SCT) in acute myeloid leukemia (AML). A large proportion of adult AML cases however lack targets for quantitative polymerase chain reaction (qPCR), hampering molecular monitoring at high sensitivity. We have recently shown that mutations suitable for MRD analysis can be identified in the vast majority of AML patients using exome sequencing and can be quantified with high sensitivity using ultra-deep targeted sequencing (1). Here we tested the clinical value of this method for molecular monitoring before and after allogeneic SCT. Material and Methods Nineteen adults and six children with AML were monitored for MRD using ultra-deep sequencing. For adult patients with AML with mutated NPM1 a total of 37 bone marrow samples obtained just before and three months after SCT were analyzed for NPM1 mutation. One of the adult patients and the six children were monitored for other, non-recurrent, leukemia-specific mutations (identified in diagnostic sample using exome sequencing as in (1)) during treatment with chemotherapy with or without allogeneic SCT. Ultra-deep sequencing was performed in multiplex on the Illumina MiSeq platform, using Truseq-library preparation with strict demultiplexing. Based on the linearity and sensitivity of the assay, MRD positivity was defined as variant allele frequency (VAF) ≥0.03%. Results from ultra-deep sequencing were compared with results from chimerism analysis (short tandem repeat-PCR/fluorescence in situ hybridization) and/or conventional MRD analysis (flow cytometry/qPCR). Results MRD positivity was detected in 9/37 bone marrow samples from the adult patients. Of these, 4 were detected pre-SCT, and 5 post-SCT. In MRD positive samples, the NPM1 mutation load ranged from VAF 0.033-1.1%. All patients relapsing (n=5) showed NPM1 MRD positivity in at least one of the samples. In patients with NPM1 MRD positivity either pre- or post-SCT, the relapse-free and overall survival were significantly shorter compared with patients with NPM1 MRD negativity at both time points (p=0.002 for both). There was no correlation between results from chimerism analysis and NPM1 mutation load. In cases that were assessed with ultra-deep sequencing for other, non-recurrent, leukemia-specific mutations, there was a high concordance between the mutation loads of different mutations assessed in the same samples. The kinetics during chemotherapy mirrored that detected with flow cytometry, with the exception that ultra-deep sequencing could detect MRD positivity in several samples determined as MRD negative with flow cytometry. In one patient where there were available blood samples before relapse of AML, ultra-deep sequencing showed MRD positivity 2 months before morphological relapse. Conclusion Molecular monitoring using targeted ultra-deep sequencing is a highly sensitive technique. The analysis can be patient-tailored and is therefore applicable to both cases with recurrent mutations and cases with non-recurrent mutations. This permits molecular monitoring and thus chances for early relapse-preventing intervention for virtually every AML patient. Reference 1. Malmberg EB, Ståhlman S, et al. Patient-tailored analysis of minimal residual disease in acute myeloid leukemia using next generation sequencing. Eur J Haematol, 2016 May 20, Epub ahead of print.

Disclosure of conflict of interest

N/A
Cord blood transplantation recapitulates fetal ontogeny with a distinct molecular signature that supports CD4+ T-cell reconstitution

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Abstract

Introduction Lymphopoiesis is thought to occur in distinct layers during different stages of ontogeny, but the role of fetal ontogeny in the unique and enhanced CD4+ biased lymphopoiesis derived from the T cells carried with the cord blood graft is not known (Fig. 1). Materials and Methods Gene expression profile (GEP) of CD4+CD45RA+CCR7+ (naïve) T cells from normal donor cord blood (n=3) and normal donor peripheral blood (n=3) were compared in 3 separate experiments. These GEPS were compared with those of reconstituting naïve CD4+ T cells at two months after T-replete CBT (n=3) and BMT (n=3). GEPS of naïve CD4+ T cells and T-regulatory cells (Tregs) from the fetal lymph nodes (18-22 weeks gestational age) and adult peripheral blood were retrieved [GSE25119] and their relationship with our experimental samples was elucidated. Results GEP of naïve CD4+ T cells from the cord blood and that of lymphocytes reconstituting following T-replete CBT were similar to the GEP of fetal CD4+ T cells but distinct to that of naïve CD4+ T cells from the peripheral blood and that of lymphocytes reconstituting after T-replete BMT (Fig. 2 a and b). Fetal ontogeny is biased towards T-regulatory function. We therefore compared GEPS of naïve CD4+ T cells and Tregs. Naïve CD4+ T cells and Tregs segregated depending on the developmental stage and T-cell type (Fig. 2 c). Thus, confirming the distinct GEP of naïve CD4+ T cells after CBT is due to recapitulation of fetal ontogeny and not due to adoption of T-reg function. In the 3 experiments comparing naïve CD4+ T cells from cord blood and peripheral blood - 288, 273 and 213 genes were differentially expressed. Sixty genes overlapped in the 3 experiments. These sixty genes are therefore likely to represent the molecular “signature” of cord blood CD4+ T cells. Cord blood T cells are in a highly proliferative state driven by the relatively lymphopenic environment of the fetus. Therefore, we speculated that some of the genes representing the naïve cord blood CD4+ T-cell “signature” may be induced by the lymphopenic environment. We attempted to identify such genes by examining those induced in steady state peripheral naïve CD4+ T cells present in the bone marrow graft after infusion into a lymphopenic transplant recipient. Nineteen of 60 overlapping genes representing the “signature” of naïve cord blood CD4+ T cells were also differentially expressed in reconstituting naïve peripheral blood CD4+ T cells following BMT. These 19 genes also remained differentially expressed in the reconstituting naïve CD4+ T cells following CBT. The up or down regulation of these 19 genes was higher in the reconstituting naïve CD4+ T cells after CBT than after BMT. In particular, genes of the T-cell receptor (TCR) signalling pathway and its transcription factor complex – activator protein-1 (AP-1) were highly upregulated in cord blood CD4+ T cells. The ligation of TCR with self-antigen presenting cells in vitro mediated enhanced proliferation of cord blood CD4+ T cells compared with peripheral blood CD4+ T cells (p<0.05). Furthermore, a small molecule inhibitor of AP-1 proportionally inhibited cord blood CD4+ T-cell proliferation (p<0.05). Conclusion Reconstituting cord blood CD4+ T cells retain the properties of fetal ontogenesis, and enhanced TCR signalling rapidly restore the unique CD4+ T-cell biased adaptive immunity after T-replete CBT.
Disclosure of conflict of interest

The authors declare no competing financial interests.