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Alterations of circulating lymphoid committed progenitor cellular metabolism after allogeneic stem cell transplantation in humans

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Lymphoid-committed $CD34^+lin^-CD10^+CD24^-$ progenitors undergo a rebound at month 3 after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in the absence of acute graft-versus-host disease (aGVHD). Here, we analyzed transcriptional programs of cell-sorted circulating lymphoid-committed progenitors and $CD34^+Lin^-CD10^-$ nonlymphoid progenitors in 11 allo-HSCT patients who had ($n = 5$) or had not ($n = 6$) developed grade 2 or 3 aGVHD and in 7 age-matched healthy donors. Major upregulated pathways include protein synthesis, energy production, cell cycle regulation, and cytoskeleton organization. Notably, genes from protein biogenesis, translation machinery, and cell cycle (CDK6) were overexpressed in progenitors from patients in the absence of aGVHD compared with healthy donors and patients affected by aGVHD. Expression of many genes from the mitochondrial oxidative phosphorylation metabolic pathway leading to ATP production were more specifically increased in lymphoid-committed progenitors in the absence of aGVHD. This was also the case for genes involved in cell mobilization such as those regulating Rho GTPase activity. In all, we found that circulating lymphoid-committed progenitors undergo profound changes in metabolism, favoring cell proliferation, energy production, and cell mobilization after allo-HSCT in humans. These mechanisms are abolished in the case of aGVHD or its treatment, indicating a persistent cell-intrinsic defect after exit from the bone marrow.

The rescue of immune competence after allogeneic hematopoietic stem cell transplantation (allo-HSCT) is linked to the recovery of de novo T-cell production in the thymus [1], which is impaired during acute graft-versus-host disease (aGVHD) and its treatments [2]. We recently reported a rebound of $CD34^+Lin^-CD10^+CD24^-$ circulating lymphoid-committed progenitors (CLPs) [3] after allo-HSCT abrogated by aGVHD [4].

Here, we studied the impact of allo-HSCT and aGVHD on gene expression of ex vivo cell-sorted circulating CLPs and $CD34^+Lin^-CD10^-$, a heterogeneous population committed to the myeloid lineage. We illustrated that circulating CLPs undergo profound changes in metabolism, favoring energy production and response to stress after allo-HSCT in humans. These mechanisms are abolished in cases of aGVHD, indicating a persistent cell-intrinsic defect [5,6].

Methods

All patients ($n = 11$) underwent non–T-cell–depleted allo-HSCT at Hôpital Saint-Louis (Paris, France) between February and November 2013 (Table 1). Five of the 11 patients developed aGVHD grade 2 or 3 [7], which responded to steroids. Healthy donors (HDs, $n = 7$) matched for age (35–63 years) and sex (4 males/3 females) were recruited at the Hôpital Saint-Louis Blood Bank. The investigation was approved by the Medical

EC and AT contributed equally to this article.

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Table 1. Patient characteristics

	Patient				Sample date	Donor				Graft		aGVHD					
	Sex	Age	CMV	Disease		Match	Sex	Age	CMV	SCS	Conditioning	Prophylaxis	Grade	Location	Onset	End	Treatment	Infections[a]
No1	F	53	Neg	AML	99	Sib	F	58	Neg	PB	Bu/Flu/ATG	CSP/MMF	0					Gram+
No2	M	53	Pos	CLL	101	Sib	M	58	Pos	PB	Bu/Flu/ATG	CSP	1	Skin	44	62		Gram+
No3	M	49	Neg	AML	104	MUD	M	26	Pos	PB	Bu/Flu/ATG	CSP/MMF	0					CMV, Gram+
No4	M	23	Pos	AA	99	Sib	M	21	Neg	BM	Cy/ATG	CSP/MTX	0					
No5	M	62	Pos	MM	101	Sib	M	61	Pos	PB	TBI 2Gy/Flu	CSP/MMF	1	Skin	78	100		Gram+
No6	M	32	Neg	Hodg	101	MUD	M	33	Pos	PB	Bu/Flu/ATG	CSP/MMF	0					Gram–, Gram+
Yes1	F	39	Neg	AML	112	Sib	F	33	Neg	PB	TBI 12Gy/Cy	CSP/MTX	3	Gut	19	84	Steroids	Gram+, Asp
Yes2	M	40	Pos	MM	113	Sib	M	36	Pos	PB	TBI 2Gy/Flu	CSP/MMF	2	Skin	48	56	Steroids	Clost.
Yes3	M	30	Neg	AML	102	Sib	F	21	Pos	PB	Bu/Cy	CSP/MTX	2	Gut	27	39	Steroids	HSV, CMV
Yes4	M	16	Pos	AML	98	MUD	M	25	Pos	PB	Bu/Cy	CSP/MTX	2	Gut	56	77	Steroids	Gram+, Gram–, CMV
Yes5	M	36	Neg	NHL	98	MUD	M	25	Neg	PB	TBI 12Gy/Cy	CSP/MTX	2	Skin	10	18	Steroids	Gram+

AA = Aplastic anemia; AML = acute myeloid leukemia; Asp = Aspergillus; ATG = antithymocyte globulin; BEAM = carmustine/etoposide/cytarabine/melphalan; Bu = busulfan; CLL = chronic lymphocytic leukemia; Clost. = Clostridium difficile; CMV = cytomegalovirus; CSP = cyclosporine; Cy = cyclophosphamide; Flu = fludarabine; Hodg = Hodgkin's lymphoma; MM = multiple myeloma; MMF = mycophenolate mofetil; MTX = methotrexate; MUD = matched unrelated donor; Neg = seronegative; NHL = non-Hodgkin's lymphoma; PB = peripheral blood; Pos = seropositive; Sample = day of sampling; SCS = stem cell source; Sib = identical sibling; Steroids = corticosteroids; TBI = total body irradiation.

[a]Only severe infections before day 100 were taken into account.

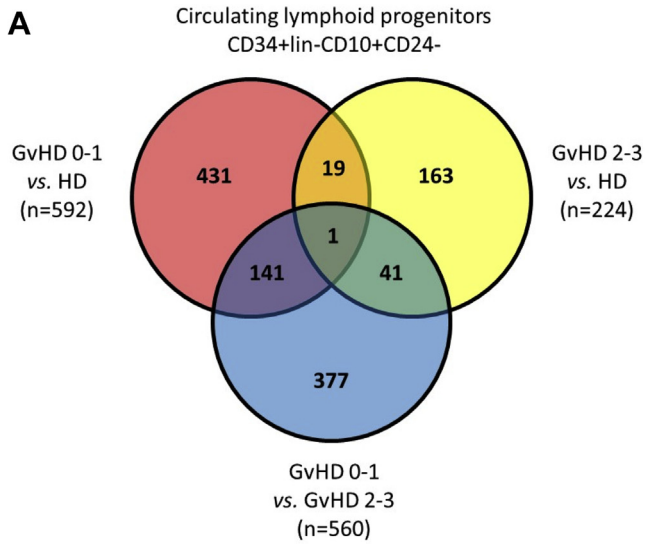
Ethics Committee of the Hôpital Saint-Louis with written informed consent obtained from all participants.

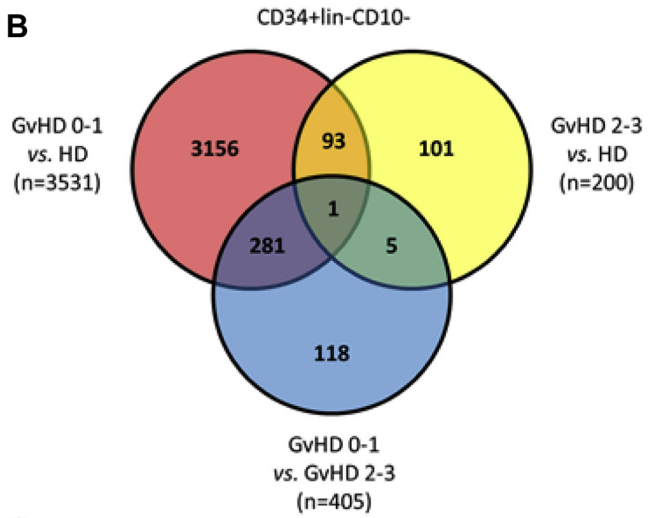
Peripheral blood mononuclear cells were first separated on lymphocyte separation medium (Eurobio, Courtaboeuf, France). At least 20×10^6 cells were stained with monoclonal mouse antihuman CD34–allophycocyanin (APC, 8G12), monoclonal mouse antihuman CD24–fluorescein isothiocyanate (FITC, ML5), and monoclonal mouse antihuman CD10–phycoerythrin cyanine 7 (PE-Cy7, HI10a). The lineage (Lin) PE-conjugated antibody cocktail contained antibodies against CD2 (RPA-2.10), CD3 (UCHT1), CD4 (RPA-T4), CD8 (RPA-T8), CD13 (WM15), CD14 (M5E2), CD15 (HI98), CD16 (3G8), CD19 (HIB19), CD20 (2H7), CD33 (WM53), CD56 (B159), and CD235a (GA-R2). All antibodies were from BD Biosciences (Le Pont de Claix, France). Five hundred $CD34^+lin^-CD10^+CD24^-$ or $CD34^+lin^-CD10^-$ cells were directly sorted into 60 µL of RLT buffer (Qiagen, Courtaboeuf, France), using a FACS ARIA II, (BD) and were immediately frozen at −80°C. Total RNA was extracted using the RNeasy Microkit (Qiagen) according to the manufacturer's instructions. RNA was quantified and qualified on a Bioanalyser 2100 (Agilent Technologies, les Ulis, France). Total RNA was amplified using the ExpressArt mRNA amplification Pico Kit (AmpTec, Hamburg, Germany) and labeled with the BioArray HighYield RNA Transcript Labeling Kit (Enzo Life Sciences, Villeurbanne, France), then hybridized to GeneChip Human Genome U133plus 2.0 arrays (Affymetrix, High Wycombe, UK). All data have been deposited in NCBI's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/) under the algorithm Maximum Rank Sum (MAXRS) [8] and normalized with global normalization Accession No. GSE75344.

A *t*-test analysis was performed for sample comparisons, and RNA was considered differentially expressed for fold changes >1.5 and p values <0.05. Pathway analyses were performed with KEGG (Kyoto Encyclopedia of Genes and Genomes) and DAVID (Database for Annotation, Visualization and Integrated Discovery). All statistical differences were expressed according to DAVID.

Results

We first compared the distribution of genes differentially expressed in the different cell populations. In cell-sorted CLPs (percentages of CLPs in $CD34^+$ cells are provided in Supplementary Figure E1 [online only, available at www.exphem.org]), 592 probes were differentially expressed between patients in the absence of aGVHD versus HDs, and 560 probes between patients without aGVHD versus those with aGVHD (540 were upregulated and 20 downregulated, $p < 0.05$, fold change > 1.5; Fig. 1A). In the control population of circulating $CD34^+Lin^-CD10^-$ nonlymphoid progenitors, 405 probes were differentially expressed between patients without aGVHD and patients with aGVHD (396 were upregulated and 9 were downregulated, $p < 0.05$, fold change > 1.5; Fig. 1B), with only 15 genes being upregulated in both $CD10^+$ and $CD10^-$ subpopulations (Supplementary Figure E2, online only, available at www.exphem.org). Of note, few genes were





C

	Compared to HD	
Pathways	GvHD 0-1	GvHD 2-3
Ribosome (*RPL3*, *RPS7*, *RPS9*, ...)	↑	=
Translation (*EIF3I*, *EIF4B*, *PAIP1*, *HNRNPD*)	↑	↓
Proteasome (*PSMA1*, *PSMB7*, *UBE2D2*)	↑	=
Cell Cycle (*CDK6*, *CDKN1B*, *E2F1*, *ANAPC16*)	↑	↓
Oxidative Phoshorylation OXPHOS (*ATP5A1*, *NDUFB3*, *SDHAP2*, *COX6B1*)	↑	↓
Fatty acid Oxidation (*LIPA*, *LPIN2*) and Glycolysis (*HK1*)	↑	↓
Leukocyte Migration (*ITGB1*, *ARHGAP8*, *ABI1*)	↑	↓
Rho GTPases (*ARHGAP32*, *KRAS*, *SRPRB*)	=	↓

Figure 1. (**A**) Number of probes differentially expressed between circulating lymphoid progenitors from patients with and without grade 1 acute GVHD (GvHD 0–1), from patients with grade 2 or 3 aGVHD (GvHD 2–3), and from healthy donors (HDs). (**B**) Number of probes differentially expressed between $CD34^{+}lin^{-}CD10^{-}$ cells from patients with and without grade 1 acute GVHD (GvHD 0–1), from patients with grade 2 or 3 aGVHD (GvHD 2–3), and from HDs. (**C**) Differences in major differentially expressed pathways between patients with (grades 2–3) and without (grades 0–1) aGVHD and from HDs in circulating lymphoid-committed progenitors.

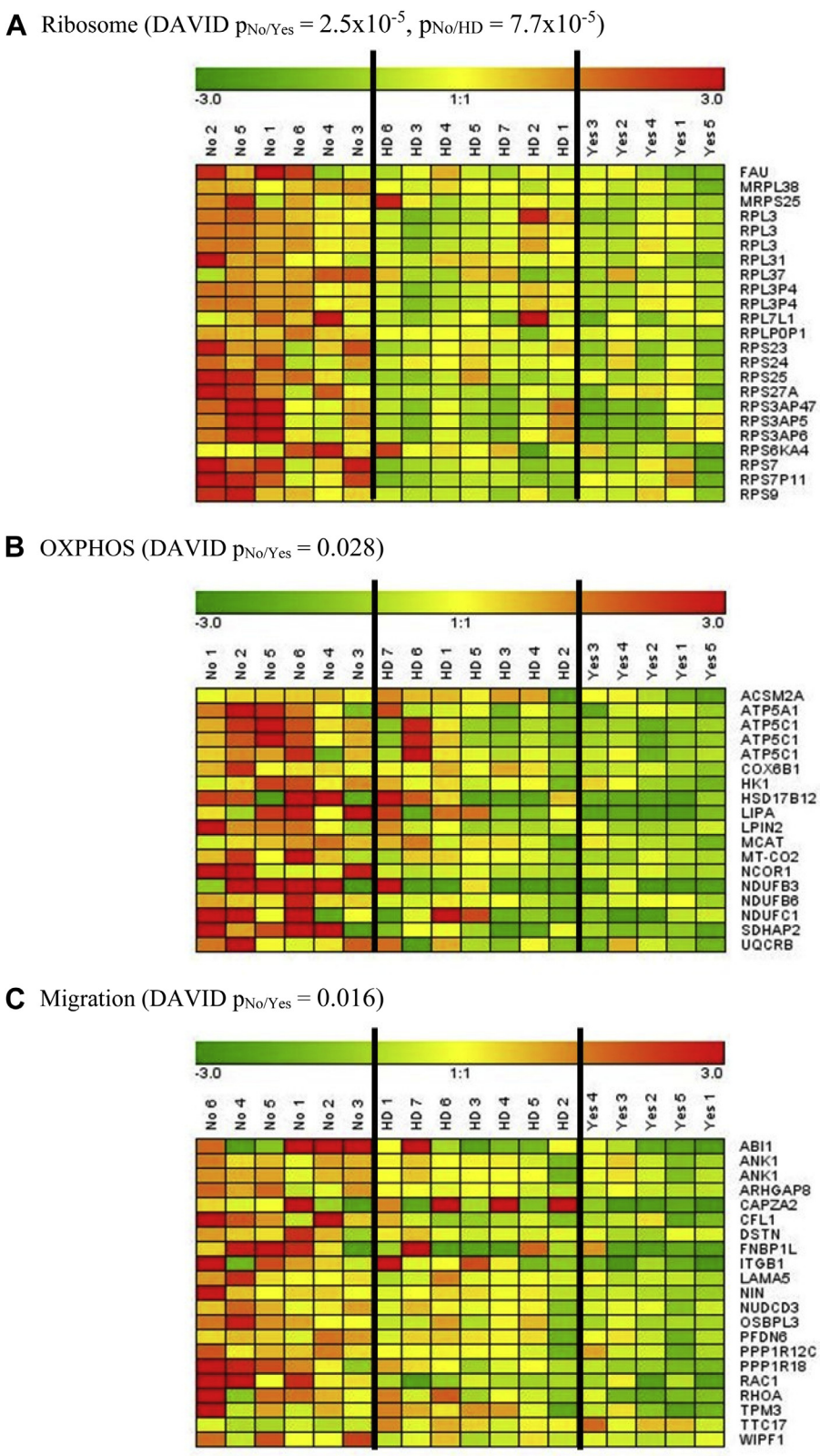


Figure 2. Expression heat map of genes from (**A**) ribosomal, (**B**) OXPHOS, or (**C**) cell migration pathways in CLPs from patients with or without grade 1 acute GVHD (No) and patients with grade 2 or 3 aGVHD (Yes) and healthy donors (HDs). Statistical significance according to DAVID is indicated between these three groups.

consistently downregulated (Supplementary Table E1, online only, available at www.exphem.org).

Major upregulated pathways included ribosome, protein synthesis, energy production, cell cycle regulation, and cytoskeleton organization (Fig. 1C). Some were affected after allo-HSCT in both progenitor populations (protein synthesis and cell proliferation), and others were affected preferentially in the CLP population (energy production and cell migration).

Genes belonging to the protein synthesis and ribosomal pathways were upregulated in both CLPs ($p = 2.5 \times 10^{-5}$; Fig. 2A) and nonlymphoid progenitors ($p = 5.7 \times 10^{-8}$) from patients without aGVHD compared with patients with aGVHD during the 3-month period preceding sample collection. Compared with HDs, these pathways were upregulated in cells from allo-HSCT patients in the absence of aGVHD and downregulated in cells from patients with aGVHD (Fig. 1C). Genes that encode subunits of eukaryotic translation initiation factors 3 (EIF3I) and 4 (EIF4B) were also upregulated in both circulating progenitor populations from patients without aGVHD, compared with cells from patients with aGVHD. Genes encoding proteins involved in mRNA biology regulating splicing (PRP6 pre-mRNA processing factor 6 homolog [PRPF6]) or stability (heterogeneous nuclear ribonucleoprotein D [HNRNPD]) were upregulated in CLPs from patients without aGVHD compared with patients with aGVHD (Supplementary Table E1). In total, the protein synthesis machinery was boosted globally after allo-HSCT and severely impaired in cases of aGVHD as compared with HDs. Accordingly, with the increased protein synthesis rate, genes that encode proteasome subunits alpha type 1 (PSMA1), alpha type 5 (PSMA5), and beta type 7 (PSMB7) or play a role in the ubiquitination process, which degrades damaged proteins, such as ubiquitin-conjugating enzyme E2D2 (UBE2D2) and ubiquitin-specific peptidase 14 (USP14), were upregulated in both circulating progenitor populations from patients without aGVHD, compared with patients with aGVHD. These genes are also upregulated in patients without aGVHD, compared with healthy donors (Fig. 1C; Supplementary Table E1). In addition, many genes encoding proteins involved in cell proliferation were upregulated in both progenitor populations in the absence of aGVHD compared with HDs and patients with aGVHD. This was the case for the cyclin-dependent kinase (CDK6), the activity of which has been linked to multipotent progenitor maintenance and thymocyte development [9] and regulation of hematopoietic stem cell quiescence [10]. These results are consistent with the rebound of progenitors observed 3 months after allo-HSCT in the absence of allogeneic aGVHD [4].

Other pathways were upregulated in the absence of aGVHD preferentially in CLPs. This was the case for the energy production pathways. The most efficient way to produce ATP is through the proton gradient produced by NAD^+ recycling in the respiratory chain or oxidative phosphorylation (OXPHOS) pathway. NAD^+ is produced from acetyl-CoA in the Krebs cycle. Acetyl-CoA can be produced by glycolysis and fatty acid oxidation (FAO). Many genes encoding for protein complexes of OXPHOS ($p = 0.028$), NADH dehydrogenases NDUFB3-B6 and -C1, succinate dehydrogenase SDHAP2, cytochrome c oxidase COX6B1, ATP synthase, H^+-transporting mitochondrial complex ATP5A1 and ATP5C1, glycolysis (hexokinase 1 HK1), and FAO enzymes (acyl-CoA synthetase medium-chain family member 2A ACSM2A) were overexpressed in the absence of aGVHD (Fig. 2B; Supplementary Table E1).

Many pathways encoding proteins implicated in cell migration ($p = 0.016$), cell adhesion ($p = 0.031$), and cytoskeleton deformation, such as abl-interactor 1 (ABI1), oxysterol binding protein-like 3 (OSBPL3), and integrin beta 1 (ITGB10), were upregulated in CLPs in the absence of aGVHD, compared with HDs and patients with aGVHD (Fig. 2C). Finally, this was also the case for genes encoding proteins implicated in Rho GTPase biology such as Rho GTPase-activating proteins 8 (ARHGAP8) and 32 (ARHGAP32) or Rho guanine nucleotide exchange factors 2 (ARHGEF2) and 7 (ARHGEF7). Rho GTPases regulate the major modes of actin polymerization and control morphogenesis, polarity, movement, and cell division [11,12]. Of note, we did not observe upregulation of genes like FASR, implicated in bone marrow (BM) immune cell death in mice, probably because their role could be restricted to the death of the stromal cells [13].

Discussion

The shift from quiescence to a proliferative state has been associated, in alloreactive T cells, with increases in mitochondrial oxygen consumption, fatty acid uptake, and oxidation [14–16]. However, hematopoietic progenitor metabolism status after allo-HSCT has not yet been studied in humans, an especially difficult task in such rare circulating cell populations. Here, we found profound alterations in metabolic pathways of circulating lymphoid progenitors, consistent with their rebound in the periphery observed 3 months after transplant [4]. The BM environment is altered during allo-HSCT by chemotherapy and conditioning, triggering stress-resistance mechanisms. Our hypothesis is that HSCs undergo a high proliferation rate, and accordingly, associated mechanisms of mRNA splicing and protein synthesis are upregulated. Transcriptions of genes from the OXPHOS and FAO pathways increase to supply the energy required for cell proliferation and migration. Genes participating in deformation and cell migration are also upregulated, favoring the exit of lymphoid progenitors from the BM and their migratory properties. After aGVHD and its treatment, none of these compensatory metabolic changes were functional. Genes encoding ribosomal proteins and FAO enzymes were downregulated

compared with healthy donors. These alterations could induce cell death and reduce proliferation and migration, participating in the delay of T-cell reconstitution during aGVHD and its treatment. These profound and multiple defects, especially in a T-cell–committed compartment, are probably acquired early in the BM-altered environment [5,6]. We demonstrated here that they persist after exit from BM, potentially affecting further lineage intrathymic differentiation.

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Authorship Contributions

SG performed research, analyzed and interpreted data, and wrote the article. IAS and JL analyzed data. RPL, SS, and GS collected data and critically reviewed the article. EC and AT designed research, analyzed and interpreted data, and wrote the article.

Conflict of interest disclosure

The authors declare no competing financial interests.

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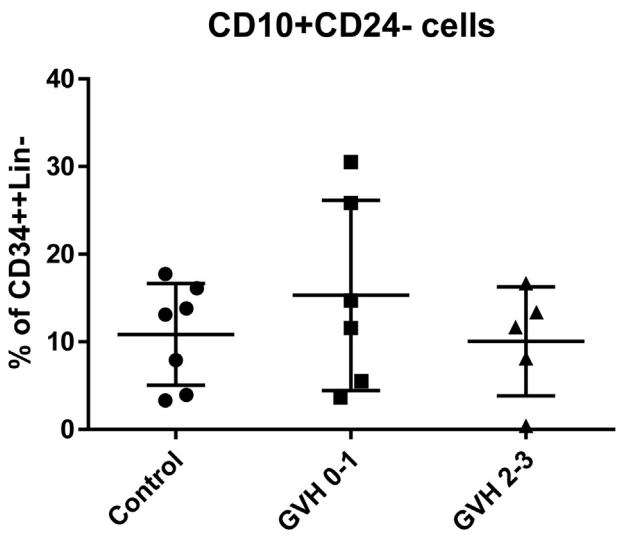
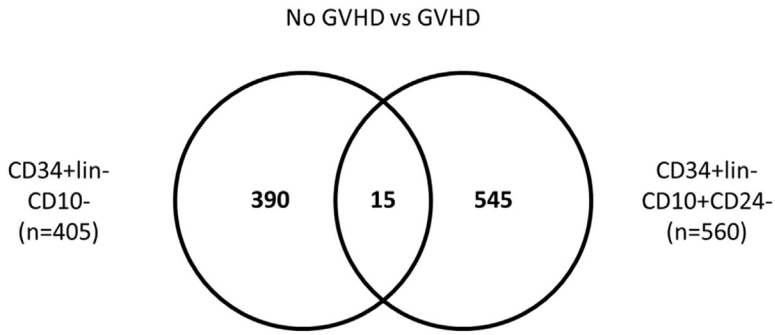


Supplementary Figure E1. Percentage of $CD10^{+}CD24^{-}$ cells among $CD34^{+}Lin^{-}$cells before sorting.

A



B

Probe ID	Ensembl Gene ID	Gene Name	Description
1553551_s_at	ENSG00000210100	J01415.5	---
1553570_x_at	ENSG00000198712	MT-CO2	mitochondrially encoded cytochrome c oxidase II [Source:HGNC Symbol;Acc:7421]
1564165_at	---	---	---
1570126_at	---	---	---
200061_s_at	ENSG00000138326	RPS24	ribosomal protein S24 [Source:HGNC Symbol;Acc:10411]
200926_at	ENSG00000186468	RPS23	ribosomal protein S23 [Source:HGNC Symbol;Acc:10410]
207559_s_at	ENSG00000147130	ZMYM3	zinc finger, MYM-type 3 [Source:HGNC Symbol;Acc:13054]
211628_x_at	ENSG00000230204	FTH1P5	ferritin, heavy polypeptide 1 pseudogene 5 [Source:HGNC Symbol;Acc:3996]
217732_s_at	ENSG00000136156	ITM2B	integral membrane protein 2B [Source:HGNC Symbol;Acc:6174]
218592_s_at	ENSG00000069998	CECR5	cat eye syndrome chromosome region, candidate 5 [Source:HGNC Symbol;Acc:1843]
221809_at	ENSG00000141084	RANBP10	RAN binding protein 10 [Source:HGNC Symbol;Acc:29285]
224927_at	ENSG00000146112	PPP1R18	protein phosphatase 1, regulatory subunit 18 [Source:HGNC Symbol;Acc:29413]
229930_at	ENSG00000229891	Z83851.1	---
234517_at	---	---	---
AFFX-M27830_5_at	---	---	---

Supplementary Figure E2. (**A**) Overlap of probes deregulated between circulating lymphoid progenitors and $CD34^{+}lin^{-}CD10^{-}$ cells from patients with and without grade 1 acute GVHD (GvHD 0–1), and patients with grade 2 or 3 aGVHD (GvHD 2–3). (**B**) List of 15 overlapping deregulated probes.

Supplementary Table E1. KEGG pathway classification of the main genes whose expression is modified by aGVHD[a]

Protein synthesis				Cell cycle		Leukocyte migration	
Ribosome		Translation		ADRA1B	1.5	ABI1	3.4
FAU	2.0	AARS	2.0	ANAPC16	4.1	ANK1	1.5
MRPL38	1.6	EIF3I	1.8	ARMC10	1.6	ANK1	1.5
MRPS25	1.8	EIF4B	1.8	CCNI	1.7	ARHGAP8	1.5
RPL3	1.7	EIF4BP6	1.7	CDC5L	1.5	CAPZA2	4.0
RPL3	1.6	HMGN3	2.4	CDK13	1.8	CFL1	2.3
RPL3	1.5	HNRNPD	2.1	CDK6	3.3	DSTN	1.5
RPL31	1.7	HNRNPD	1.9	CDKN1B	2.2	FNBP1L	3.0
RPL37	1.6	MIR1224	1.7	CKS2	−1.6	ITGB1	4.4
RPL3P4	1.6	PAIP1	1.6	CNOT7	2.9	LAMA5	1.5
RPL3P4	1.5	PRPF38B	1.6	DIXDC1	1.5	NIN	1.8
RPL7L1	1.7	PRPF40A	5.7	DUT	2.3	NUDCD3	1.5
RPLP0P1	1.5	PRPF6	1.5	E2F1	1.5	OSBPL3	1.9
RPS23	1.9	PUM1	1.9	MALAT1	2.5	PFDN6	1.5
RPS24	1.6	PUM1	1.6	MALAT1	2.3	PPP1R12C	1.6
RPS25	1.7	PUM2	1.5	MPLKIP	2.0	PPP1R18	2.6
RPS27A	1.8	SSB	3.8	PBXIP1	1.7	RAC1	3.2
RPS3AP47	2.6	TAF15	2.2	PPP4C	1.7	RHOA	2.9
RPS3AP5	2.5	TOP1MT	18.1	PRKCA	1.7	TPM3	2.0
RPS3AP6	1.9	TRIO	1.7	PTPN2	2.6	TTC17	−1.5
RPS6KA4	1.9	YARS	1.6	RFC1	1.9	WIPF1	1.5
RPS7	1.9	ZCRB1	2.0	TPR	1.6	Rho GTPases	
RPS7P11	1.7			Proteasome		ARHGAP32	1.5
RPS9	1.6			PSMA1	1.9	ARHGDIB	1.7
Energy production				PSMA5	3,5	ARHGEF2	1.9
OXPHOS		FAO and glycolysis		PSMB7	1.5	ARHGEF7	1.7
ACSM2A	1.6	LIPA	1.8	PSMC2	3.1	CYTH4	2.0
ATP5A1	2.0	LPIN2	4.3	UBE2D2	3.6	GDI2	4.7
ATP5C1	2.9	MCAT	6.0	UBE2E1	2.5	KRAS	5.8
ATP5C1	2.7	HK1	1.9	UBE2G2	1.7	NRAS	2.3
ATP5C1	1.7	HSD17B12	1.6	UFD1L	1.6	RAB18	17.0
COX6B1	1.7	NCOR1	1.9	URM1	1.9	RAB18	2.7
MT-CO2	1.9			USP14	1.8	RALB	2.0
NDUFB3	5.3			USP22	7.8	RAP1A	2.5
NDUFB6	1.5			USP4	1.8	SRPRB	1.5
NDUFC1	3.2						
SDHAP2	3.8						
UQCRB	2.1						

[a]Fold changes correspond to the difference between $CD34^{+}lin^{-}CD10^{+}CD24^{-}$ lymphoid-committed progenitors from allo-HSCT patients without (grades 0–1) aGVHD and those from patients with grade 2–3 aGVHD.