

## T-cell receptor- $\alpha$ repertoire of CD8<sup>+</sup> T cells following allogeneic stem cell transplantation using next-generation sequencing

Cornelia S. Link-Rachner,<sup>1,2</sup> Anne Eugster,<sup>2</sup> Elke Rücker-Braun,<sup>1</sup> Falk Heidenreich,<sup>1,3</sup> Uta Oelschlägel,<sup>1</sup> Andreas Dahl,<sup>2,4</sup> Christian Klesse,<sup>3</sup> Matthias Kuhn,<sup>5</sup> Jan Moritz Middeke,<sup>1</sup> Martin Bornhäuser,<sup>1,2</sup> Ezio Bonifacio<sup>2</sup> and Johannes Schetelig<sup>1,3</sup>

<sup>1</sup>Medizinische Klinik und Poliklinik I, Universitätsklinikum Carl Gustav Carus, TU Dresden; <sup>2</sup>DFG Research Center for Regenerative Therapies Dresden, TU Dresden; <sup>3</sup>DKMS Clinical Trials Unit, Dresden; <sup>4</sup>BIOTEchnology Center, TU Dresden and <sup>5</sup>Institut für Medizinische Informatik und Biometrie (IMB), Medizinische Fakultät der TU Dresden, Germany

©2019 Ferrata Storti Foundation. This is an open-access paper. doi:10.3324/haematol.2018.199802

Received: June 22, 2018.

Accepted: September 25, 2018.

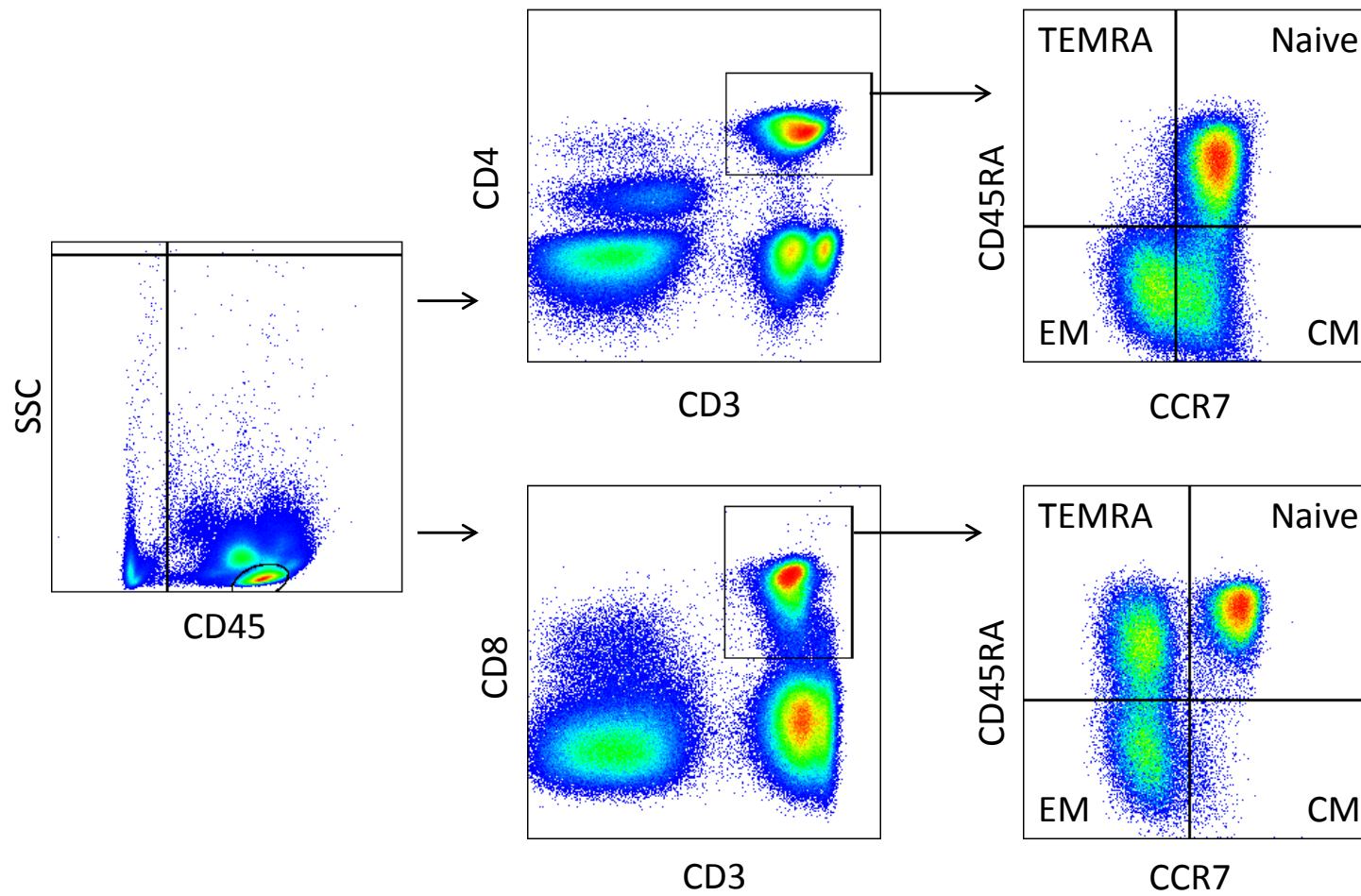
Pre-published: September 27, 2018.

Correspondence: CORNELIA S. LINK-RACHNER

cornelia.link@uniklinikum-dresden.de

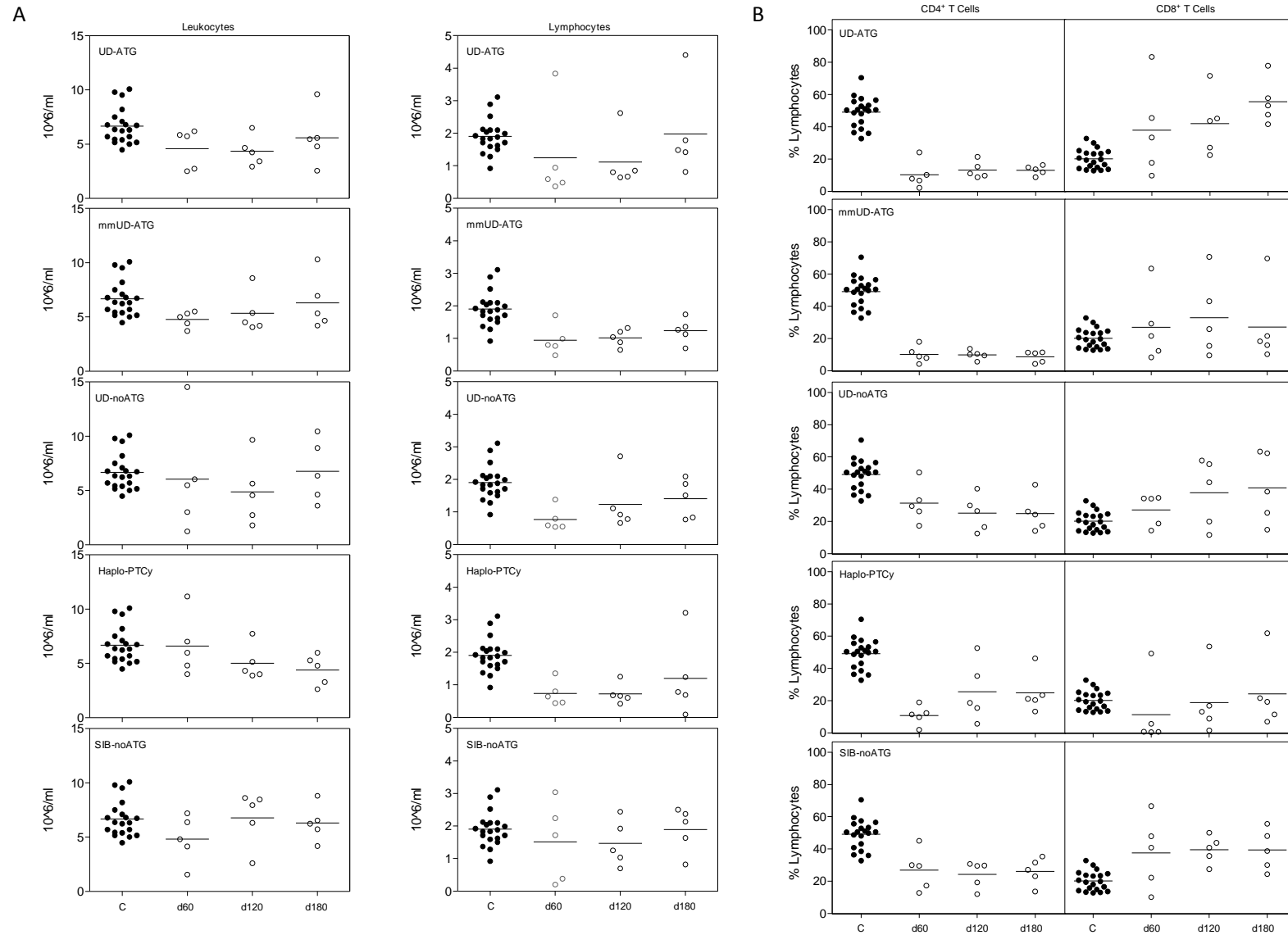
---

Suppl. Figure 1



**Supplemental Figure 1: FACS Gating.** Flow cytometry was performed to characterize T cell subsets. Naive T cells were defined as NAIVE (CCR7<sup>+</sup>CD45RA<sup>+</sup>), central memory (CM, CCR7<sup>+</sup>CD45RA<sup>-</sup>), effector memory (EM, CCR7<sup>-</sup>CD45RA<sup>-</sup>) and terminally differentiated effector memory T cells (TEMRA, CCR7<sup>-</sup>CD45RA<sup>+</sup>).

## Suppl. Figure 2



**Supplemental Figure 2: (A) Leukocytes and lymphocytes.** Immune reconstitution of whole leukocytes (left) and lymphocytes (right). **(B) CD8<sup>+</sup> and CD4<sup>+</sup> T cells.** Five groups with five patients each (UD-ATG, mmUD-ATG, UD-noATG, Haplo-PTCy and SIB-noATG) were analysed separately in comparison to a reference population (C).

Suppl. Table 1

Subset	day SCT	SIB-noATG			
		ID#	Reads	Clonotypes	No cells
N	60	TCR_002	1,940,036	7,105	72,821
N	60	TCR_011	713,547	2,064	65,075
N	60	TCR_024	1,264,879	14,684	158,132
N	60	TCR_040	n.a.	n.a.	n.a.
N	60	TCR_063	9,478	305	2,000
N	180	TCR_002	1,023,953	5,992	115,743
N	180	TCR_011	2,648,640	22,121	321,250
N	180	TCR_024	1,389,988	6,662	174,000
N	180	TCR_040	118,614	1,295	7,444
N	180	TCR_063	295,201	4,168	70,997
M	60	TCR_002	9,779,405	4,409	1,000,000
M	60	TCR_011	16,493,432	9,104	1,000,000
M	60	TCR_024	5,471,026	10,881	373,307
M	60	TCR_040	21,945,112	3,372	1,000,000
M	60	TCR_063	14,377,359	3,075	1,000,000
M	180	TCR_002	12,724,714	4,094	1,000,000
M	180	TCR_011	18,127,834	7,522	1,000,000
M	180	TCR_024	13,931,550	8,540	1,000,000
M	180	TCR_040	11,031,826	2,371	1,000,000
M	180	TCR_063	12,317,051	2,908	1,000,000
N	Don	TCR_002	57,697	2,873	6,741
N	Don	TCR_011	683,397	4,956	72,901
N	Don	TCR_024	1,315,974	8,429	142,370
N	Don	TCR_040	154,266	968	43,177
N	Don	TCR_063	97,207	870	40,000
M	Don	TCR_002	14,617,055	4,153	1,000,000
M	Don	TCR_011	13,388,336	4,328	681,521
M	Don	TCR_024	5,826,943	4,386	441,483
M	Don	TCR_040	38,709,214	13,985	328,815
M	Don	TCR_063	16,364,935	3,458	1,000,000

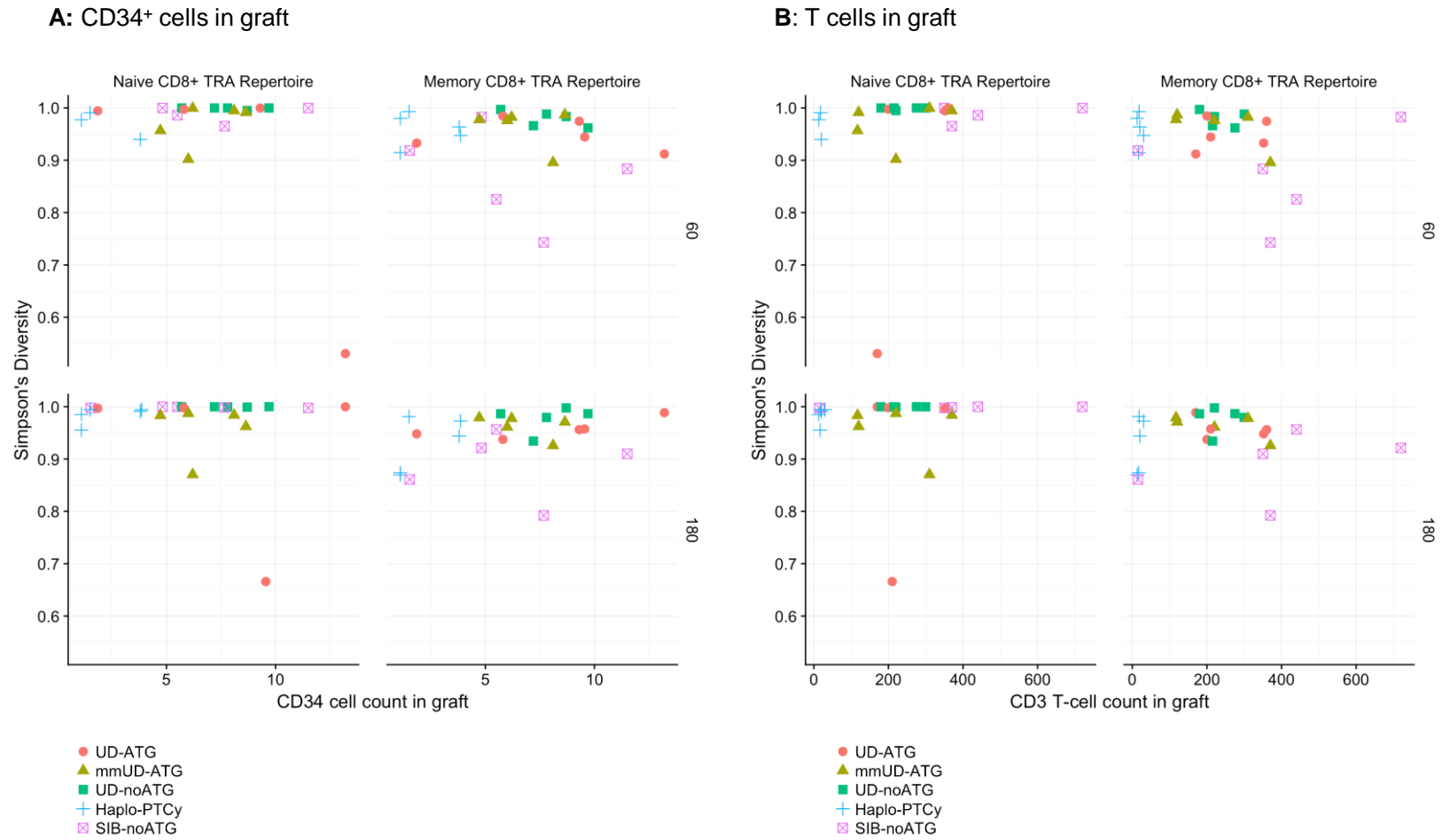
**Supplemental Table 1: Sequencing results.** Obtained TRA reads and clonotypes for patients in the SIB-noATG group and their donors (Don). Patient samples were taken on day 60 (60) and day 180 (180) following transplantation. Total numbers of TRA reads and clonotypes for each patient for the naive (N) and memory (M) CD8<sup>+</sup> TRA repertoires as well as the total number of analysed cells are shown. Samples that failed quality control after sequencing were excluded from analyses and are defined as not available (n.a.).

**Suppl. Table 2**

Subset	day	SCT	UD-ATG				mmUD-ATG				UD-noATG				Haplo-PTCy			
			ID#	Reads	Clonotypes	No. of cells	ID#	Reads	Clonotypes	No. of cells	ID#	Reads	Clonotypes	No. of cells	ID#	Reads	Clonotypes	No. of cells
N	60		TCR_001	454,406	1,627	52,173	TCR_013	1,960,865	6,199	88,886	TCR_005	5,633,166	18,481	201,209	TCR_012	n.a.	n.a.	n.a.
N	60		TCR_003	374,443	6,666	40,461	TCR_038	4,849	231	1,798	TCR_008	161,559	1,937	23,281	TCR_014	1,270	200	335
N	60		TCR_023	128,453	1,488	14,382	TCR_048	31,045	270	3,037	TCR_017	6,496,190	22,953	644,328	TCR_026	n.a.	n.a.	n.a.
N	60		TCR_041	69,591	1,066	26,126	TCR_054	87,105	891	8,000	TCR_019	10,066,215	87,347	575,537	TCR_036	8,185	396	3,108
N	60		TCR_056	n.a.	n.a.	n.a.	TCR_055	49,267	1,054	5,560	TCR_027	1,724,416	26,241	245,215	TCR_049	14,319	764	4,282
N	180		TCR_001	1,966,016	14,313	165,552	TCR_013	8,140,898	12,929	355,112	TCR_005	18,407,371	63,508	1,000,000	TCR_012	208,903	4,249	9,184
N	180		TCR_003	n.a.	n.a.	n.a.	TCR_038	30,680	801	2,406	TCR_008	1,000,621	6,342	103,073	TCR_014	1,419	202	238
N	180		TCR_023	785,371	5,140	49,000	TCR_048	83,412	334	24,000	TCR_017	15,451,086	18,167	580,001	TCR_026	1,742,467	10,500	163,000
N	180		TCR_041	99,529	1,834	10,000	TCR_054	23,910	608	3,440	TCR_019	7,946,959	107,277	1,000,000	TCR_036	82,652	1,643	7,875
N	180		TCR_056	628,909	679	92,000	TCR_055	50,921	1,216	2,112	TCR_027	2,022,917	9,209	152,000	TCR_049	70,623	1,269	12,000
M	60		TCR_001	8,225,606	6,815	1,000,000	TCR_013	16,307,546	11,786	1,000,000	TCR_005	18,381,678	15,367	1,000,000	TCR_012	2,432,031	3,785	1,000,000
M	60		TCR_003	5,298,664	5,212	383,525	TCR_038	596,050	1,863	95,162	TCR_008	3,986,855	5,562	187,602	TCR_014	18,116	382	95,162
M	60		TCR_023	6,338,572	6,554	590,498	TCR_048	18,873,775	6,843	1,000,000	TCR_017	12,128,971	20,084	1,081,490	TCR_026	12,836,244	5,292	1,000,000
M	60		TCR_041	18,798,673	5,837	1,000,000	TCR_054	22,327,833	4,330	1,000,000	TCR_019	14,624,500	16,619	872,311	TCR_036	1,623,753	3,420	1,000,000
M	60		TCR_056	12,136,892	3,378	1,000,000	TCR_055	8,215,980	7,664	295,000	TCR_027	14,588,247	3,569	1,000,000	TCR_049	235,759	1,179	295,000
M	180		TCR_001	12,493,617	4,099	1,000,000	TCR_013	14,439,589	13,759	1,000,000	TCR_005	13,188,971	9,087	1,000,000	TCR_012	12,633,706	6,294	1,000,000
M	180		TCR_003	16,291,702	11,843	1,000,000	TCR_038	4,656,672	2,087	267,000	TCR_008	17,028,133	19,866	1,000,000	TCR_014	211,590	693	82,469
M	180		TCR_023	12,446,408	7,949	1,000,000	TCR_048	8,713,436	4,448	1,000,000	TCR_017	6,334,346	5,386	852,793	TCR_026	15,829,436	10,924	1,000,000
M	180		TCR_041	17,986,250	3,990	737,000	TCR_054	31,937,572	10,372	715,000	TCR_019	9,149,322	24,063	1,000,000	TCR_036	5,859,926	4,137	366,000
M	180		TCR_056	14,779,589	4,293	1,000,000	TCR_055	9,425,752	7,215	630,000	TCR_027	19,873,218	16,289	1,000,000	TCR_049	10,781,324	6,819	517,000

**Supplemental Table 2: Sequencing results.** Obtained TRA reads and clonotypes for patients in the four groups (UD-ATG, mmUD-ATG, UD-noATG and Haplo-PTCy) on day 60 (60) and day 180 (180) following transplantation. Total numbers of TRA reads and clonotypes for each patient for the naive (N) and memory (M) CD8<sup>+</sup> TRA repertoires as well as the total number of analysed cells are shown. Samples that failed quality control after sequencing were excluded from analyses and are defined as not available (n.a.).

### Suppl. Figure 3



**Supplemental Figure 3: Graft cell counts.** Simpson's diversity index ( $D_s$ ) on day 60 (60) and day 180 (180) of the naive and memory TRA repertoires of each patient following transplantation. Repertoire diversity is mapped in relation to CD34<sup>+</sup> cells (**A**) and T cells (**B**) counted in the stem cell graft.