Non-myelotoxic chemicals as a preparatory regimen for hematopoietic stem cell gene therapy

Mehmet Emin ŞEKER, Özgür Doğuş EROL, Burcu PERVİN, Gerard WAGEMAKER, Nico P. van TIL, Fatima AERTS-KAYA

Supplemental data

Table S1. Primer forward and reverse sequences

Gene	Forward sequence	Reverse sequence	Amplicon size
Human RAG2co	ACCATCTTCAGCAAGGACAG	CACGTAGATCTTGTCGCTCAC	150
mouse Rag2	ATTCAACCAGGCTTCTCACTT	TGCAGGCTTCAGTTTGAGATG	138
mouse Gapdh	CATCACTGCCACCCAGAAGAC	TGACCTTGCCCACAGCCTTG	122
HIV U3	CTGGAAGGGCTAATTCACTC	GGTTTCCCTTTCGCTTTCAG	274
WPRE	TTGCTTCCCGTACGGCTTTC	AGCAAACACAGAGCACACCA	116
mouse Sry	CATCGGAGGGCTAAAGTGTCAC	TGGCATGTGGGTTCCTGTCC	103

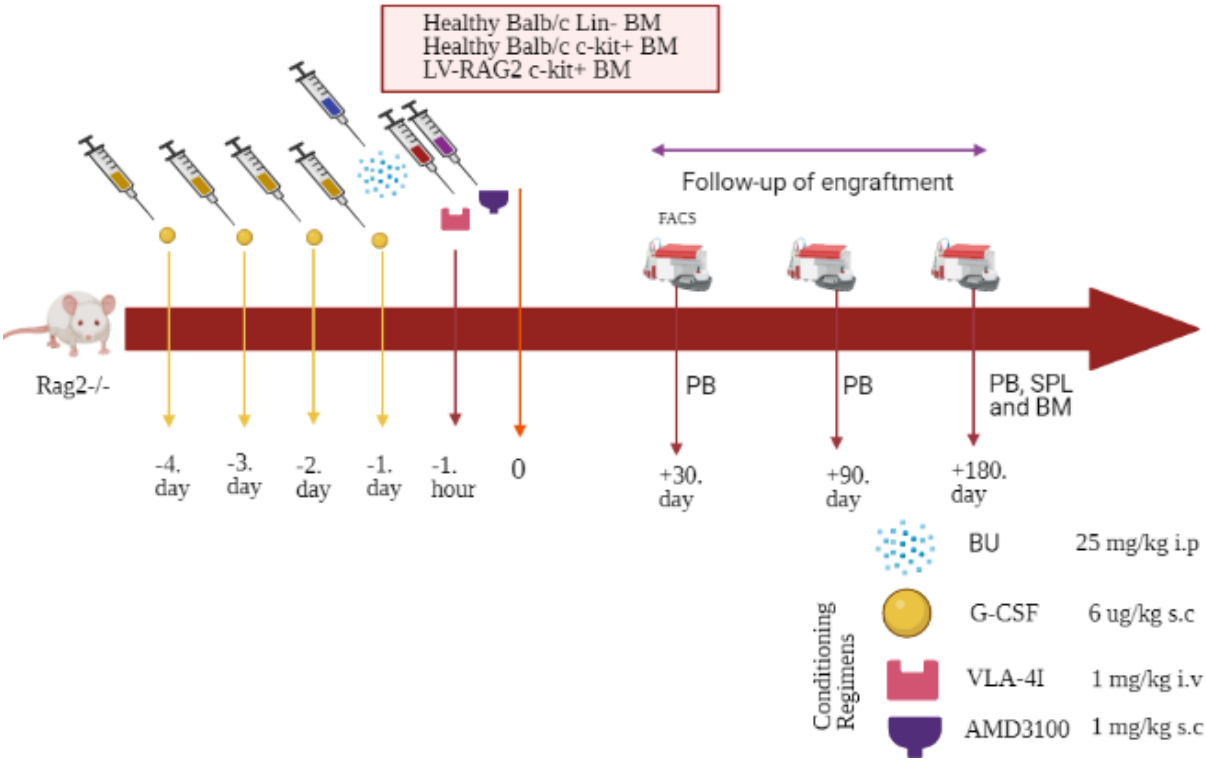

Figure S1. Conditioning regimens and follow up of RAG2 mice after transplantation. Mice were divided into 4 groups: 1) 25 mg/kg BU i.p., day -1 (Sigma, #BCBS8240V); 2) 6 ug/kg/d G- CSF s.c. from day -4 to -1 (Dong-a St Co. Ltd, Leucostim 30); 3) 1 mg/kg VLA-4I i.v. (Selleck Chemicals, #S7198) 1 hour before transplant and; 4) 1 mg/kg AMD3100 s.c. (Cayman, #10011332) 1 hour before transplant. PB white blood cell numbers were manually counted before and after treatment, just before transplantation. Each group contained 4-6 mice. Analyses were performed from peripheral blood at 1 and 3 months, and from peripheral blood, bone marrow and spleen at 6 months. PB: peripheral blood, BM: Bone marrow, SPL: Spleen Rag2: Recombination activating gene 2 LSK: $Lin^{-}Sca1^{+}c\text{-}Kit^{+}$, BU: Busulfan, G-CSF: Granulocyte colony-stimulating factor, VLA-4I: very late antigen 4 inhibitors. Figures were created using the Biorender program.

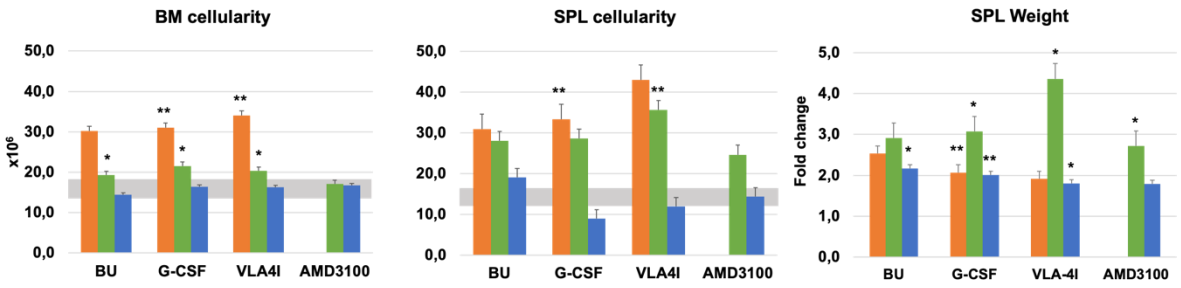

Figure S2. **Bone marrow, spleen cellularity and weight.** At sacrifice 6 months post-transplantation, BM (left) and spleen cellularity (middle) were increased in mice transplanted with healthy Lin- BM cells (orange bars, $p<0.01$) and healthy c-kit+ BM cells (green bars, $p<0.05$). Cellularity remained largely unchanged in mice transplanted with lentivirally transduced RAG2 c-Kit+ BM cells (blue bars, $p>0.05$) in comparison to untreated RAG2 mice (horizontal grey background lines depict the average BM and SPL cellularity ± standard deviation for untreated RAG2 mice). No significant differences were found between methods of conditioning. All transplanted mice showed an increase in spleen weight in comparison to pre-transplant weights (right). PB: peripheral blood; BM: bone marrow; SPL: Spleen; Rag2: Recombination activating gene 2; BU: Busulfan; G-CSF: Granulocyte colony-stimulating factor; VLA-4I: Very late antigen 4 inhibitors; AMD3100. * $p<0.05$; ** $p<0.01$ in comparision to untreated RAG2 mice.

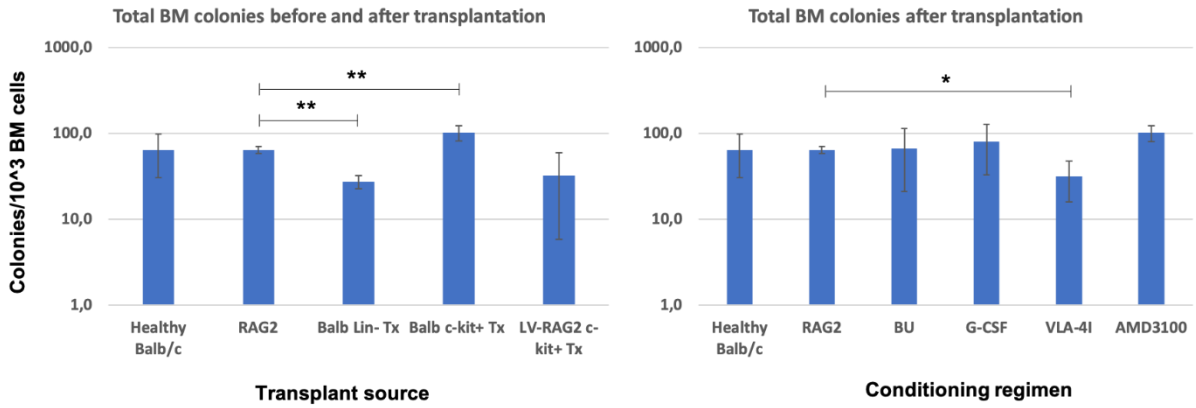

Figure S3. Total BM colonies before and after transplantation. Total BM colonies (CFU-GEMM, CFU-GM and BFU-E) obtained 6 months after transplantation were counted and normalized to colonies/10^3 plated BM cells. Mice in all groups were pooled to assess the effect of transplant source (left) or conditioning regimen (right). Statistical analyses were performed in comparison to untreated RAG2 mice. No differences in total numbers of colonies was found between healthy Balb/c and untreated RAG2 mice. After transplantation significant differences were found between mice transplanted with healthy Balb/c Lin- BM cells and untreated RAG2 mice (** $p<0,01$) and mice treated with healthy Balb/c c-Kit+ BM cells and untreated RAG2 mice (** $p<0,01$). No significant differences were observed between conditioning regimens, however, total colony numbers were somewhat lower in the VLA-4I treated group, in comparison to untreated RAG2 mice (* $p<0,05$).

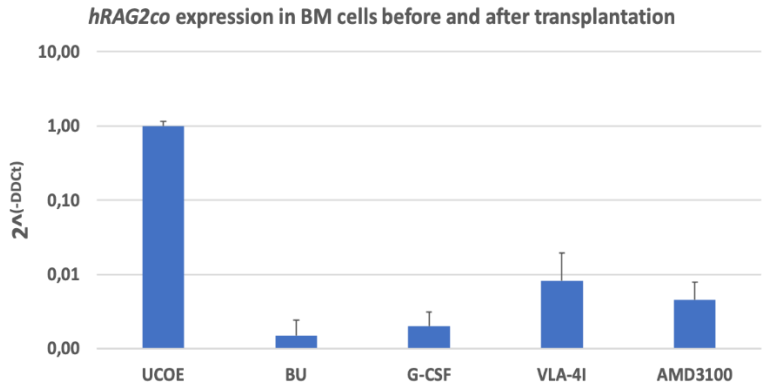

Figure S4. Expression of hRAG2co in bone marrow of transplanted animals. RAG2-/- c-Kit+ BM cells were transduced with LV-UCOE-hRAG2co at an MOI of 30. hRAG2co expression was measured directly after transduction, but before transplantation (UCOE) and 6 months after transplantation in total BM cells of mice were pre-treated with BU, G-CSF, VLA-4I and AMD3100. Data are given as mean ± standard deviation (n=3).

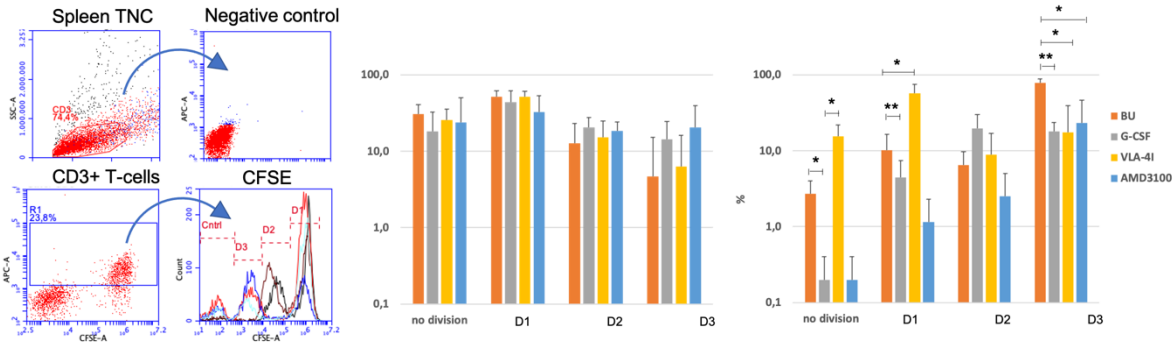

Figure S5. Spleen cell proliferation assay. RAG2 mice pre-treated with BU, G-CSF, VLA-4I or AMD3100 were transplanted with healthy Balb/c (middle) or LV-hRAG2co transduced (right) c-Kit+ BM cells and sacrificed at 6 months post transplantation. Total spleen nucleated cells (TNCs) were collected, stained with CFSE and cultured in presence of PHA-P and hIL-2 for 4 days. After 4 days cells were stained with anti-CD3 to be able to gate for the T-cell population and cell divisions were measured using CFSE staining. With every division, the intensity of the CFSE staining decreases, therefore the peak at the most right represents cells that only divided once (D1), the next peak represents 2 divisions (D2) and the next peak 3 divisions (D3). The peak at the left represents unstained cells. * $p<0.05$, ** $p<0.01$.

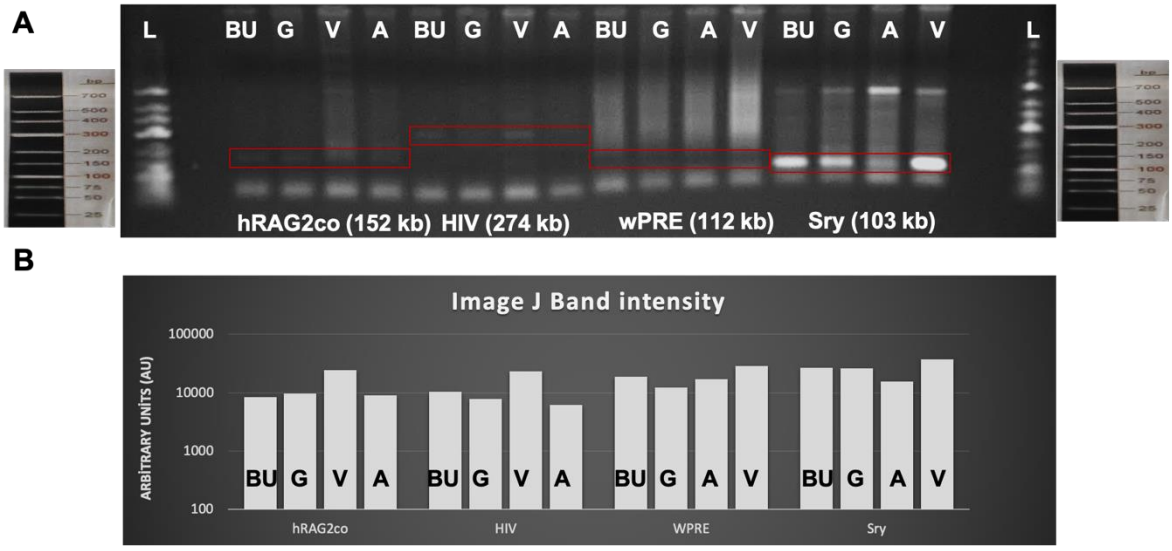

Figure S6. Assessment of engraftment of donor-derived cells by PCR. A: Genomic PCR showing the presence of the male Sry gene, indicating robust overall engraftment in all transplantation groups and the presence of the transgene hRAG2co, as well as construct-related HIV and wPRE sequences in spleen total nucleated cells obtained from female mice at 6 months post-transplantation, confirming permanent integration into the genome and engraftment of LV-hRAG2co transduced male Rag2$^{-/-}$ c-Kit+ BM cells after conditioning with Busulfan (BU) or non-myelotoxic pre-treatment with G-CSF (G), VLA-4I (V) or AMD3100 (A). B: Differences in band intensity (arbitrary units) of the above genomic PCR, calculated using ImageJ, confirming engraftment of all treatment groups, with highest engraftment of gene transduced cells in the VLA-4I group, despite low overall engraftment, while treatment with AMD3100 shows low engraftment of gene transduced cells, despite highest overall engraftment.