Quest for the cure: towards improving hematopoietic stem cell based lentiviral gene therapy

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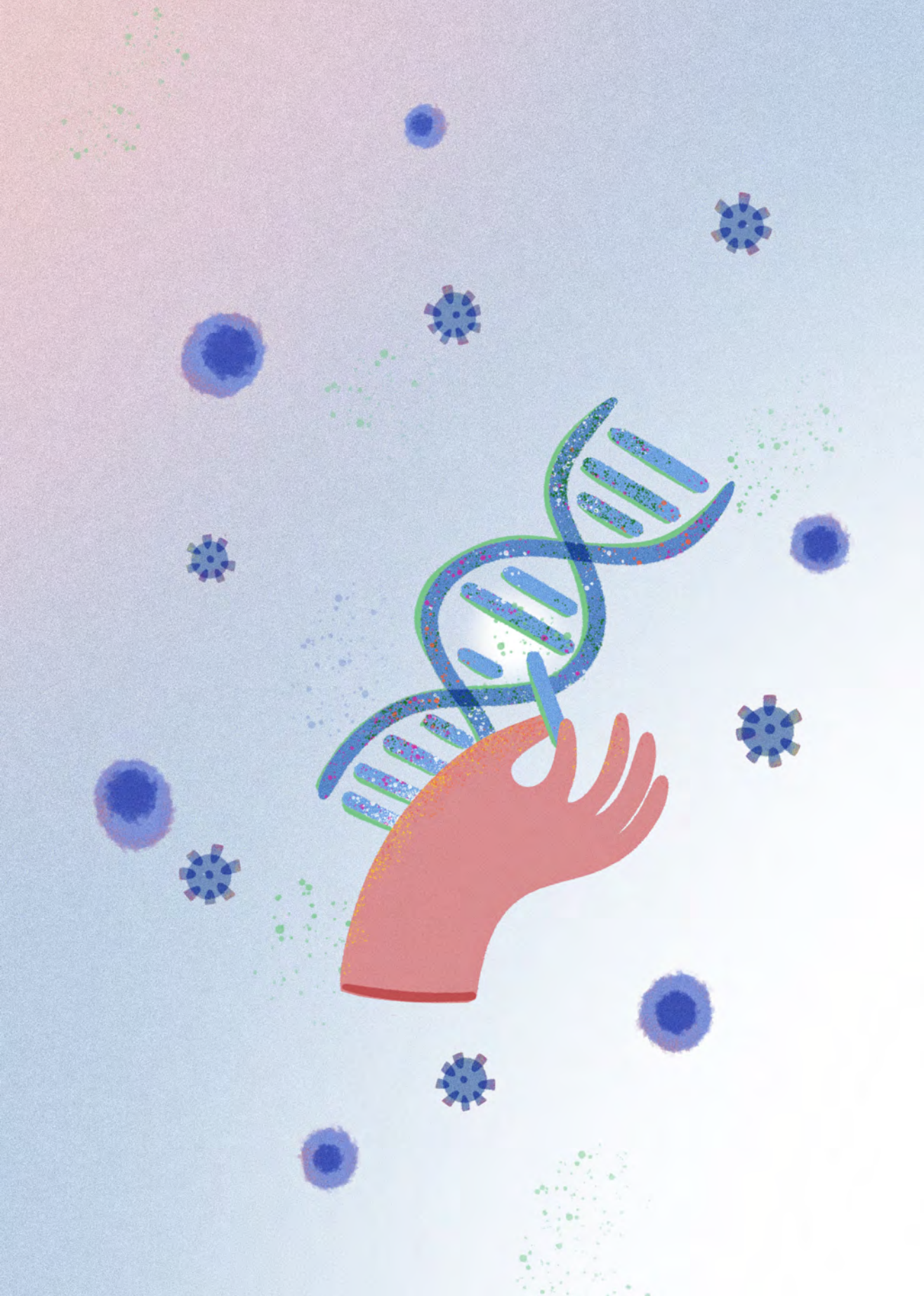

Chapter 3

IL3 has a detrimental effect on hematopoietic stem cell self-renewal in transplantation setting

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Abstract

Ex vivo expansion and maintenance of long-term hematopoietic stem cells (LT-HSC) is crucial for stem cell-based gene therapy. A combination of Stem Cell Factor (SCF), Thrombopoietin (TPO), FLT3 Ligand (FLT3) and Interleukin 3 (IL3) cytokines has been commonly used in clinical settings for expansion of $CD34^+$ from different sources, prior to transplantation. To assess the effect of IL3 on repopulating capacity of cultured $CD34^+$cells we employed the commonly used combination of STF, TPO and FILT3 with or without IL3. Expanded cells were transplanted into NSG mice followed by secondary transplantation. Overall, this study shows that IL3 leads to lower human cell engraftment and repopulating capacity in NSG mice, suggesting a negative effect of IL3 on HSC self-renewal. We therefore recommend omitting IL3 from HSC-based gene therapy protocols.

Introduction

Long-term hematopoietic stem cells (LT-HSCs) give rise to all blood cells during the life time of an individual, through a process termed hematopoiesis. HSCs have the unique capacity of self-renewal and multipotency, while progenitors have a more defined path for specific lineage development [1-3]. HSCs from different sources have been used in the stem cell and gene therapy field. However, a main challenge of the limited number of HSCs that can be enriched from patient remains. Therefore, *ex vivo* expansion or maintenance of HSCs has become crucial to have long term clinical benefit of transplanted cells. HSCs undergo symmetrical and asymmetrical cell divisions *in vivo*. Asymmetric division yields differentiating cells, whereas symmetric cell division leads to expansion of HSCs, in numerical terms. Therefore, approaches that will result in symmetric stem cell division and self-renewal without further differentiation are required for *ex vivo* expansion [4]. Different combinations of cytokines and additive have been assessed to expand HSCs *in vitro*; however, limited success has been reported in clinical setting, due to cell proliferation and cell cycle activation which subsequently leads to lower *in vivo* engraftment potentials of input cells [5-8]. Currently, a combination of Stem cell factor (SCF), Flt3 ligand (FLT3), Thrombopoietin (TPO) and interleukin 3 (IL3) are used as the key factors for proliferation and maintenance of hematopoietic stem and progenitor cells (HSPCs) during *ex vivo* culturing systems in clinical settings [9,10]. Most clinical protocols for HSC based gene therapy use this four-cytokine cocktail, which we also use in our ongoing clinical trial for RAG1-SCID [11].

SCF and FLT3 were shown to induce essential signals for HSC development [12-15]; TPO has been known to stimulate proliferation and expansion of HSCs [13]. On the other hand, controversial results have been reported for the effect of IL3 on expansion of LT-HSCs. IL3 is known for its myelopoietic effect, proliferation regulation of myeloid progenitors and differentiation of granulocyte-monocyte progenitors into basophile in murine bone marrow [16-19]. Most of these studies could not confirm the clinical importance of IL3 in the long-term repopulation capacity of expanded LT-HSCs [20-22]. Therefore, we set out to evaluate the effect of IL3 on long term repopulating capacity of expanded HSCs in two cytokine combinations of SCF +TPO+FLT3 (STF) and SCF+TPO+FLT3+IL3 (STF+IL3) for a short period of time (4 days), as is often used in gene therapy protocols. Our data show that IL3 strongly reduces the repopulation capacity of cultured hematopoietic stem cells.

Ex vivo expansion of hematopoietic stem cells in cytokine-supplemented medium

To address the effect of IL3 on *ex vivo* expansion of $CD34^+$ cells, enriched $CD34^+$ cells from cord blood were cultured in X-vivo medium supplemented with a clinically used cytokine cocktail STF+IL3 (SCF, TPO, FLT3, and IL3) and STF (SCF, TPO and FLT3) for 4 days.

$CD34^+$ are known to constitute a heterogenous population [23], therefore we performed extensive flowcytometry analysis to identify different subsets of hematopoietic stem cells and progenitors (HSPCs) at the start of culturing (referred as day 0) and after 4 days of culture. Composition of different subsets of HSPCs were identified by flowcytometry analysis based on well-known markers [24] (Supplementary Figure.1A).

Total nucleated cells (TNCs) during 4 days of culture in STF and STF+IL3 cytokine cocktail were expanded 3 and 8-fold respectively (Figure 1B). Although the increase of TNC was achieved during 4 days of culture, LT-HSCs were largely lost in both cytokine cocktails (Figure 1C). On the other hand, other progenitors, specifically Multi-Lymphoid Progenitors (MLPs), Common Myeloid Progenitors (CMP) and Granulocyte Macrophage Progenitor (GMP) were expanded up to 10-fold in both culture conditions compared to day 0 (Figure 1D, Supplementary Figure S1C). Over-all, the addition of IL3 to the STF cytokine cocktail appears to benefit the numerical expansion of hematopoietic progenitors *in vitro*.

IL3 reduces human engraftment in NSG mice

To assess the repopulating capacity of expanded cells *in vivo*, cultured cells in both conditions were transplanted into NSG mice. The NSG mouse model is currently the accepted gold standard for assessing multilineage potential and self-renewal of human HSCs, because reliable *in vitro* assays to address stem cell functionality are absent.

Peripheral blood (PB) was collected from week 4 to week 20 to monitor human engraftment over time. Mice were sacrificed at week 20 post transplantation; expanded cells in STF-supplemented medium show significantly higher human chimerism (% $huCD45^+$) in PB compared to the mice that received cells expanded in STF+IL3 (Figure 2B). Flow plots of harvested BM cells show significant higher chimerism and absolute count of $huCD45^+$ cells in the STF group in comparison with the STF+IL3 group (Figure 2C-D). Consistently lower human chimerism in STF+IL3 group was also observed in spleen and thymus (Figure 2E). BM cells of engrafted

mice were further analysed for the presence of mature hematopoietic cell populations. Cells within the huCD45$^+$ gate were analysed for myeloid, lymphoid and CD34$^+$ populations (Supplementary Figure S2A).

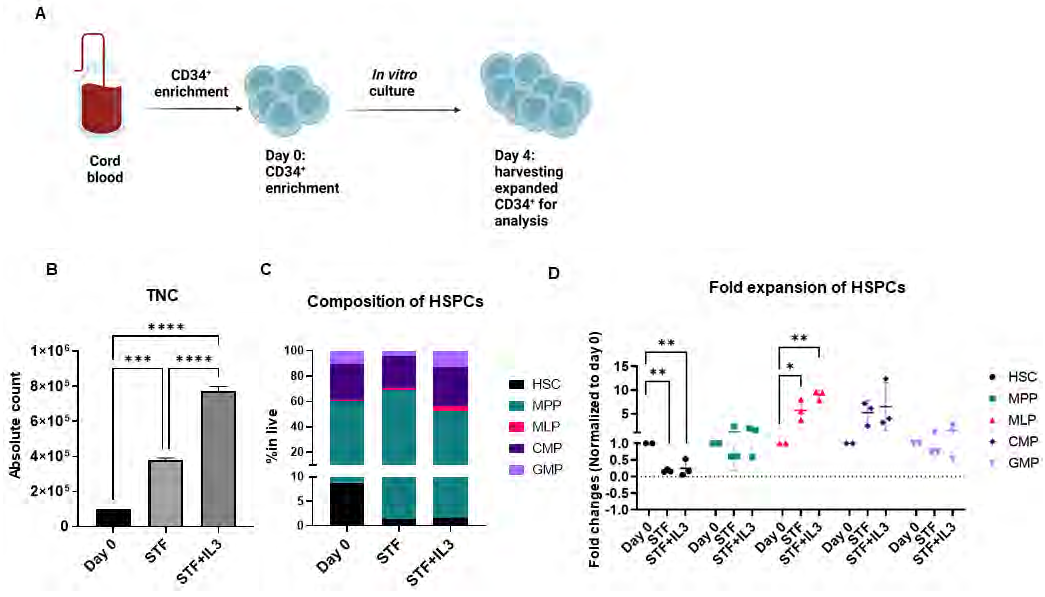

Figure 4. Human CD34$^+$ cells were expanded in cytokine-supplemented medium. (A) Schematic experimental design. (B) Total Nucleated cells (TNC) increased between -three and eight- fold in presence of cytokine after 4 days of culture. (C) Stacked bar graph shows composition of Hematopoietic Stem cells and Progenitors (HSPCs) on day 0 (after enrichment) and after 4 days of culture with the indicated cytokine cocktails. (D) Dot plot shows fold changes of expanded HSPCs on day 4 compared to day 0.

Mice from the STF+IL3 group show lower percentages of CD34$^+$ and CD3$^+$ cells within CD45$^+$ BM cells in contrast to the STF group, while no significant changes in other lineages were observed between the two groups (Figure 2F). Although a lower percentage of CD34$^+$ was observed in the STF+IL3 group, no clear difference of HSC and MPP between groups were observed. Similar analyses were performed for other organs at week 20 post transplantation. No significant differences among populations were observed except for CD3$^+$ and CD4$^+$CD8$^+$ DP thymocytes, which show a decrease in the STF+IL3 group (Supplementary Figure 2B-2D). Therefore, IL3 addition during the expansion phase of CD34$^+$ HSPCs results in substantially lower overall engraftment in NSG mice, whereas the composition of the different hematopoietic lineages remain largely unaffected.

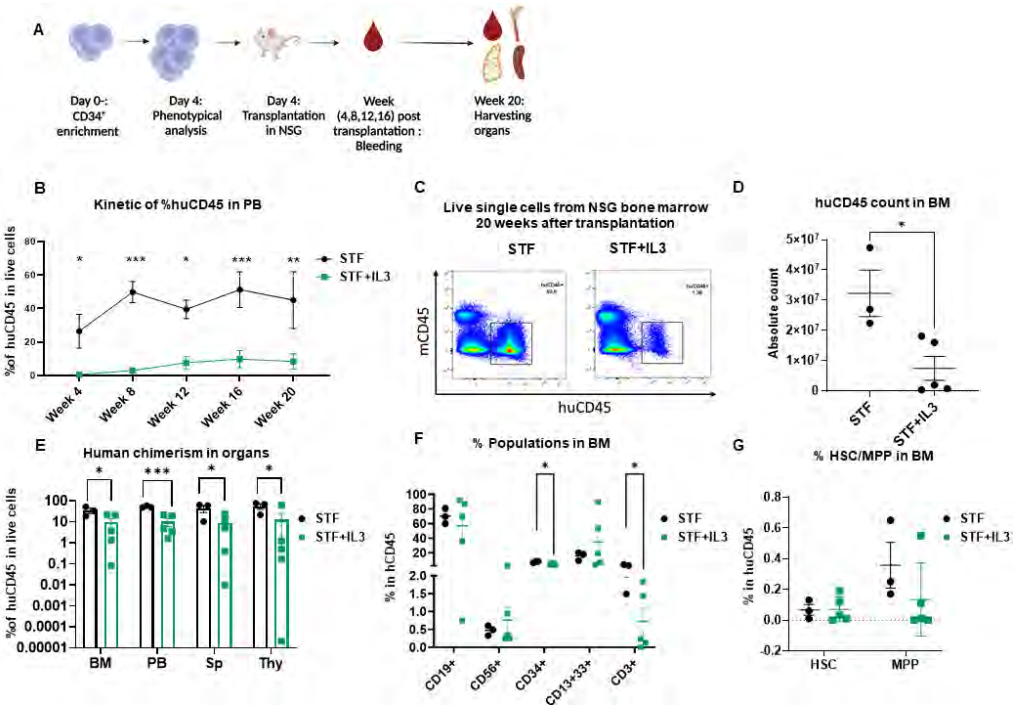

Figure 5. IL3 has an adverse effect on human engraftment in NSG mice. (A) Schematic experimental design. (B) Human CD45 engraftment in PB over time (n=3-5; *p<0,05, **p<0,01 and ***p<0,001, 2-way Anova test with multiple comparison). (C) Representative flow cytometry plots of bone marrow cells harvested from mice that received expanded cells. (D) Absolute count of human $CD45^+$ cells in bone marrow after 20 weeks of transplantation. (n=3-5; *p<0,05, **p<0,01 and ***p<0,001, unpaired t-test). (E) Percentage of human CD45 cell engraftment in different organs (BM= Bone marrow, PB= Peripheral Blood, Sp=Spleen, Thy=Thymus) in primary recipient NSG mice 20 weeks after transplantation (n=3-5; *p<0,05, **p<0,01 and ***p<0,001, unpaired t-test). (F) Percentage of $CD19^+$, $CD56^+$, $CD34^+$, $CD13^+33^+$and $CD3^+$ populations within the $huCD45^+$ BM population (n=3-5; *p<0,05, **p<0,01 and ***p<0,001, unpaired t-test). (G) Percentage of hematopoietic stem cells (HSC) and Multipotent progenitor (MPP) in BM of primary recipient NSG mice 20 weeks after transplantation (n=3-5; *p<0,05, **p<0,01 and ***p<0,001, unpaired t-test).

IL3 reduces the repopulation capacity of human hematopoietic stem cells in mice

To further evaluate the effect of IL3 on the long-term repopulation capacity of LT-HSCs, a secondary transplantation was performed. In this experiment, BM cells from primary recipients were pooled and transplanted into secondary recipient mice (Figure 3A). Extensive flow cytometry analysis was performed at the time of transplantation to assess the absolute count and percentages of human CD45$^+$, CD34$^+$, HSC and MPP transplanted into each mouse per group (Supplementary Figure S3B-D).

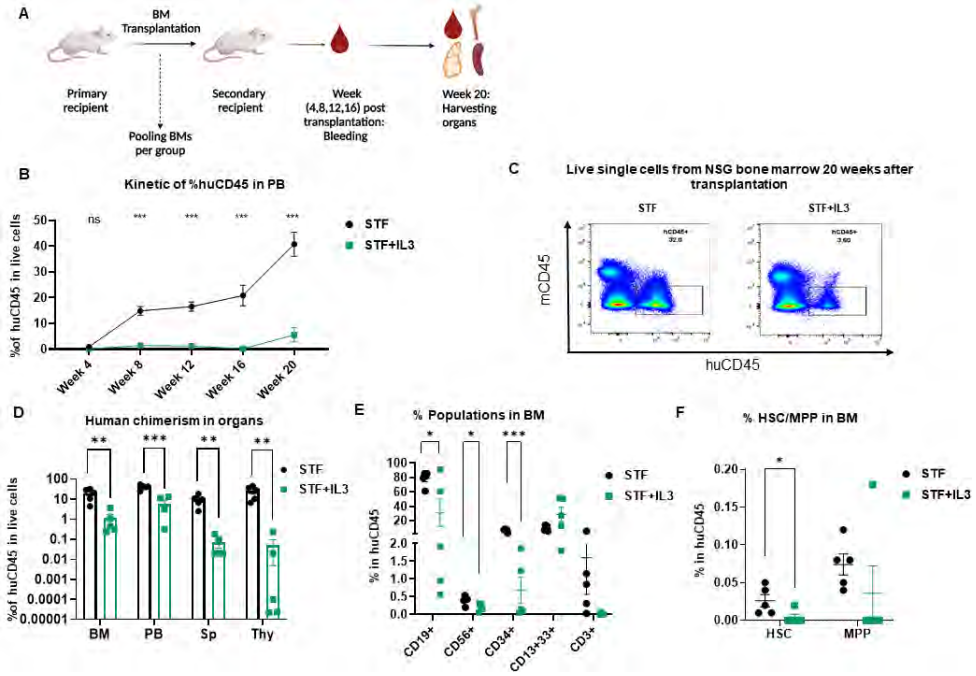

Figure 6. Negative effect of IL3 on repopulating capacity of hematopoietic stem cells revealed by secondary transplantation. (A) Schematic picture of secondary transplantation. (B) Human engraftment in PB (n=5; *p<0,05, **p<0,01 and ***p<0,001, 2-way Anova test with multiple comparison). (C) Flow plots of harvested BM cells show human engraftment. (D) Percentage of human CD45+ cells in different organs (BM= Bone marrow, PB= Peripheral Blood, Sp=Spleen, Thy=Thymus) from secondary recipient NSG mice 20 weeks after transplantation (n=5; *p<0,05, **p<0,01 and ***p<0,001, unpaired t- test). (E) Percentage of CD19$^+$, CD56$^+$, CD3$^+$, CD34$^+$ and CD13$^+$33$^+$ populations within the huCD45$^+$ BM population from secondary recipient NSG mice 20 weeks post transplantation (n=5; *p<0,05, **p<0,01 and ***p<0,001, unpaired t-test).

Human engraftment was assessed in PB from week 4 up to 20 weeks post transplantation. Also, after the secondary transplantation, IL3 addition led to a significant decrease of human chimerism in PB (Figure 3B) and similarly in BM, spleen and thymus (Figure 3C-D); this decrease is more pronounced than the primary transplantation. Cells within the huCD45$^+$ gate were further analysed for the presence of different lineages development in BM. IL3-treated stem cells differentiated much worse into CD19$^+$, CD34$^+$, CD56$^+$ cells, presumably due to lower self-renewal of HSC populations in BM (Figure 3E-F).

Similar analyses were performed in PB, spleen and thymus of secondary recipient mice 20 weeks post transplantation. The group STF+IL3 showed significantly lower lymphoid development in PB, thymus and spleen, whereas the CD13+CD33$^+$ myeloid population is unaffected (Supplementary Figure S3E-G). Taken together, IL3 affects not only the human engraftment in NSG mice, but reveals a specific defect in the reconstitution of the lymphoid compartment upon secondary transplantation.

Discussion

Different cytokine combinations have been studied for expansion and maintenance of hematopoietic stem and progenitor cells *in vitro* [13,22,25,26]. However, some of these studies have failed to consider the clinical implications of cytokine combinations on expansion and engraftment of CD34$^+$ progenitor cells, as mainly *in vitro* assays such as colony forming assays or long-term culture initiating cell assays have been used to assess the functionality of LT-HSCs.

Based on early findings, combinations of Stem cell factor (SCF), Flt3 ligand (FLT3), Thrombopoietin (TPO) and Interleukin 3 (IL3) have been established as crucial factors for supporting HSC and progenitors proliferation and maintenance during *in vitro* culturing systems particularly in clinical settings [9,10]. However, controversial results have been reported on the effect of IL3 on *ex vivo* expansion of long-term hematopoietic stem cells.

IL3 supports myelopoiesis and proliferation of lineage-committed progenitors in culture [27,28]. Some studies reported negative effects of IL3 on engraftment of expanded murine cells in bone marrow of recipient mice [21,29]. *Piacibello et al* showed lower engraftment of cultured human CD34$^+$ cells with IL3; however, repopulating capacity of engrafted cells in secondary transplantation in NSG was not determined [20]. In this study we assess the effect of IL3 on maintenance of *in vivo* repopulating capacity of *ex vivo* expanded human CD34$^+$ cells from cord blood.

Collectively, our results from the primary and secondary transplantations in NSG mice show that *ex vivo* expanded CD34$^+$ cells in the presence of IL3 fail to preserve their repopulation and self-renewal capacity, suggesting the negative effect of IL3 on the maintenance of LT-HSCs in culture. Moreover, the data from the secondary transplantations reveals the detrimental effect of IL3 on long-term development of the lymphoid lineage (B, T, NK cells) in BM, PB, spleen and thymus.

Inclusion of IL3 in a clinical protocol for expansion of CD34$^+$ for gene therapy purposes results in an increase of total CD34$^+$ cells; however, the quality of LT-HSCs is compromised in favor of the expansion of lineage-committed progenitors such as MLP, CMP and GMP. Following earlier studies, our findings emphasize the importance of maintenance and expansion of LT-HSCs for gene and cell therapy purposes. Thus, optimal culture condition for *ex vivo* expansion of HSPCs that retain their self-renewal capacity is essential for HSC-based gene therapy, especially for diseases such as SCID, XLA and others that primarily target the lymphoid compartment. Based on these considerations we propose omitting IL3 for HSC-based gene therapy aimed at restoring the lymphoid linages and thereby regenerating adaptive immunity.

Materials and Methods

Human Cells and CD34$^+$ enrichment

Human cord blood was obtained after informed consent from Leiden University Medical Center. Mononuclear Cells (MNCs) were obtained from cord blood density centrifugation using Ficoll-Amidotrizoaat. CD34$^+$ cells were positively selected using the human CD34 UltraPure MicroBead Kit (Miltenyi Biotec) according to the manufacturer's protocol. 5.2 CD34$^+$ cell culture

100,000 enriched CD34$^+$ cells/ml were cultured in X-vivo15 medium (Lonza) supplemented with recombinant huSCF (300ng/ml), huTPO (100ng/ml), huFLT3 (100ng/ml) and huIL3 (20ng/ml) (from Miltenyi Biotec). After 4 days of culture, expanded cells were harvested and counted using a nucleocounter 3000 (Chemometic) for subsequent immunophenotyping and transplantation in mice.

Mice

NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were purchased from Charles River (France). All animal experiments were approved by the Dutch Central Commission for Animal experimentation (Centrale Commissie Dierproeven, CCD).

Primary and secondary transplantations into NSG mice

For the primary transplantations, *ex vivo* expanded $CD34^+$ cells (50,000 total nucleated cells per mouse) in Iscove's Modified Dulbecco's Medium (IMDM) without phenol red (Gibco) were transplanted by tail vein injection into pre-conditioned recipient NSG mice (n=5). For the secondary transplantations, bone marrow (BM) cells from primary recipient mice from each group were pooled and $1/7^{th}$ of the pooled cells were injected into pre-conditioned secondary recipient NSG mice (n=5). 6-8-week-old recipient mice were conditioned with two consecutive doses of 25mg/kg Busulfan (Sigma-Aldrich) (48h and 24h prior transplantation). Mice used for transplantation were kept under specific pathogen-free conditions. The first four weeks after transplantation mice were fed with additional DietGel recovery food (Clear H_2O) and antibiotic water containing 0.07 mg/mL Polymixin B (Bupha Uitgeest), 0.0875 mg/mL Ciprofloxacin (Bayer b.v.). Peripheral blood (PB) from the mice was drawn by tail vein puncture every 4 weeks until the end of the experiment. At the end of the experiment, PB, thymus, spleen and BM were harvested. Mice were euthanized via CO_2-asphyxiation.

Flow cytometry

Ex vivo cultured cells were stained with the live/dead marker Zombie (Biolegend) according to the manufacturer's instruction. Subsequently, cells were stained with antibodies listed in Supplementary Table S1, and incubated for 30 min at 4°C in the dark in FACS Buffer (PBS pH 7.4, 0.1% azide, 0.2% BSA). Single cell suspensions from murine BM, thymus and spleen were prepared by squeezing the organs through a 70 µM cell strainer (BD Falcon). Erythrocytes from PB and spleen were lysed in NH_4Cl (8,4 g/L)/$KHCO_3$ (1 g/L). Single cell suspensions were counted and stained as described above. All cells were measured on an Aurora spectral flow cytometer (Cytek). The data was analyzed using FlowJo software (Tree Star).

Statistics

Statistics were calculated and graphs were generated using GraphPad Prism9 (GraphPad Software). Statistical significance was determined by standard one/two-tailed Mann-Whitney U tests, unpaired t-tests and ANOVA tests (*$p<0.05$,**$p < 0.01$, ***$p < 0.001$ and ****$p<0.0001$).

Supplementary Materials

The following are available online at https://www.mdpi.com/article/10.3390/ijms232112736/s1.

Author Contributions

P.T preformed experiments, data collection, data analysis and drafted the original manuscript. K.C.B supported mice experiments, and editing of the manuscript. B.A.E.N, S.A.V and M.C.J.A.E assisted with mice experiments. M.L.H provided cord blood for this study. K.P.O and F.J.T.S provided supervision and revision of the manuscript. All authors have read and agreed to the final version of manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

References

1. Körbling, M.; Estrov, Z. Adult Stem Cells for Tissue Repair — A New Therapeutic Concept? *The New England journal of medicine* **2003**, *349*, 570-582, doi:10.1056/NEJMra022361.
2. Copelan, E.A. Hematopoietic Stem-Cell Transplantation. *The New England journal of medicine* **2006**, *354*, 1813-1826, doi:10.1056/NEJMra052638.
3. Kondo, M.; Wagers, A.J.; Manz, M.G.; Prohaska, S.S.; Scherer, D.C.; Beilhack, G.F.; Shizuru, J.A.; Weissman, I.L. Biology of hematopoietic stem cells and progenitors: implications for clinical application. *Annual review of immunology* **2003**, *21*, 759-806, doi:10.1146/annurev.immunol.21.120601.141007.
4. Morrison, S.J.; Kimble, J. Asymmetric and symmetric stem-cell divisions in development and cancer. *Nature* **2006**, *441*, 1068-1074, doi:10.1038/nature04956.
5. Bhatia, M.; Bonnet, D.; Kapp, U.; Wang, J.C.Y.; Murdoch, B.; Dick, J.E. Quantitative Analysis Reveals Expansion of Human Hematopoietic Repopulating Cells After Short-term Ex Vivo Culture. *Journal of Experimental Medicine* **1997**, *186*, 619-624, doi:10.1084/jem.186.4.619 %J Journal of Experimental Medicine.
6. Shpall, E.J.; Quinones, R.; Giller, R.; Zeng, C.; Baron, A.E.; Jones, R.B.; Bearman, S.I.; Nieto, Y.; Freed, B.; Madinger, N., et al. Transplantation of ex vivo expanded cord blood. *Biology of Blood and Marrow Transplantation* **2002**, *8*, 368-376, doi:https://doi.org/10.1053/bbmt.2002.v8.pm12171483.
7. Peled, T.; Mandel, J.; Goudsmid, R.N.; Landor, C.; Hasson, N.; Harati, D.; Austin, M.; Hasson, A.; Fibach, E.; Shpall, E.J., et al. Pre-clinical development of cord blood-derived progenitor cell graft expanded ex vivo with cytokines and the polyamine copper chelator tetraethylenepentamine. *Cytotherapy* **2004**, *6*, 344-355, doi:https://doi.org/10.1080/14653240410004916.
8. de Lima, M.; McMannis, J.; Gee, A.; Komanduri, K.; Couriel, D.; Andersson, B.S.; Hosing, C.; Khouri, I.; Jones, R.; Champlin, R., et al. Transplantation of ex vivo expanded cord blood cells using the copper chelator tetraethylenepentamine: a phase I/II clinical trial. *Bone Marrow Transplantation* **2008**, *41*, 771-778, doi:10.1038/sj.bmt.1705979.
9. Metcalf, D. Hematopoietic cytokines. *Blood* **2008**, *111*, 485-491, doi:10.1182/blood-2007-03-079681 %J Blood.
10. Hofmeister, C.C.; Zhang, J.; Knight, K.L.; Le, P.; Stiff, P.J. Ex vivo expansion of umbilical cord blood stem cells for transplantation: growing knowledge from the hematopoietic niche. *Bone Marrow Transplantation* **2007**, *39*, 11-23, doi:10.1038/sj.bmt.1705538.
11. Garcia-Perez, L.; van Eggermond, M.; van Roon, L.; Vloemans, S.A.; Cordes, M.; Schambach, A.; Rothe, M.; Berghuis, D.; Lagresle-Peyrou, C.; Cavazzana, M., et al. Successful Preclinical Development of Gene Therapy for Recombinase-Activating Gene-1-Deficient SCID. *Molecular therapy. Methods & clinical development* **2020**, *17*, 666-682, doi:10.1016/j.omtm.2020.03.016.

12. Shah, A.; Smogorzewska, E.; Hannum, C.; Crooks, G. Flt3 ligand induces proliferation of quiescent human bone marrow CD34+CD38- cells and maintains progenitor cells in vitro. *Blood* **1996**, *87*, 3563-3570, doi:10.1182/blood.V87.9.3563.bloodjournal8793563 %J Blood.
13. Petzer, A.L.; Zandstra, P.W.; Piret, J.M.; Eaves, C.J. Differential cytokine effects on primitive (CD34+CD38-) human hematopoietic cells: novel responses to Flt3-ligand and thrombopoietin. *Journal of Experimental Medicine* **1996**, *183*, 2551-2558, doi:10.1084/jem.183.6.2551 %J Journal of Experimental Medicine.
14. McKenna, H.; de Vries, P.; Brasel, K.; Lyman, S.; Williams, D. Effect of flt3 ligand on the ex vivo expansion of human CD34+ hematopoietic progenitor cells. *Blood* **1995**, *86*, 3413-3420, doi:10.1182/blood.V86.9.3413.bloodjournal8693413 %J Blood.
15. Hoffman, R.; Tong, J.; Brandt, J.; Traycoff, C.; Bruno, E.; Srour, E.F.; Gordon, M.S.; Mcguire, B.W.; Mcniece, I. The in vitro and in vivo effects of stem cell factor on human hematopoiesis. *Stem Cells* **1996**, *11*, 76-82, doi:10.1002/stem.5530110813 %J Stem Cells.
16. Yonemura, Y.; Ku, H.; Hirayama, F.; Souza, L.M.; Ogawa, M. Interleukin 3 or interleukin 1 abrogates the reconstituting ability of hematopoietic stem cells. *Proceedings of the National Academy of Sciences of the United States of America* **1996**, *93*, 4040-4044, doi:doi:10.1073/pnas.93.9.4040.
17. Gammaitoni, L.; Bruno, S.; Sanavio, F.; Gunetti, M.; Kollet, O.; Cavalloni, G.; Falda, M.; Fagioli, F.; Lapidot, T.; Aglietta, M., et al. Ex vivo expansion of human adult stem cells capable of primary and secondary hemopoietic reconstitution. *Experimental Hematology* **2003**, *31*, 261-270, doi:https://doi.org/10.1016/S0301-472X(02)01077-9.
18. Knobel, K.M.; McNally, M.A.; Berson, A.E.; Rood, D.; Chen, K.; Kilinski, L.; Tran, K.; Okarma, T.B.; Lebkowski, J.S. Long-term reconstitution of mice after ex vivo expansion of bone marrow cells: differential activity of cultured bone marrow and enriched stem cell populations. *Exp Hematol* **1994**, *22*, 1227-1235.
19. Ohmori, K.; Luo, Y.; Jia, Y.; Nishida, J.; Wang, Z.; Bunting, K.D.; Wang, D.; Huang, H. IL-3 induces basophil expansion in vivo by directing granulocyte-monocyte progenitors to differentiate into basophil lineage-restricted progenitors in the bone marrow and by increasing the number of basophil/mast cell progenitors in the spleen. *Journal of immunology (Baltimore, Md. : 1950)* **2009**, *182*, 2835-2841, doi:10.4049/jimmunol.0802870.
20. Piacibello, W.; Gammaitoni, L.; Bruno, S.; Gunetti, M.; Fagioli, F.; Cavalloni, G.; Aglietta, M. Negative influence of IL3 on the expansion of human cord blood in vivo long-term repopulating stem cells. *Journal of hematotherapy & stem cell research* **2000**, *9*, 945-956, doi:10.1089/152581600750062408.
21. Peters, S.O.; Kittler, E.L.; Ramshaw, H.S.; Quesenberry, P.J. Ex vivo expansion of murine marrow cells with interleukin-3 (IL-3), IL-6, IL-11, and stem cell factor leads to impaired engraftment in irradiated hosts. *Blood* **1996**, *87*, 30-37.
22. Piacibello, W.; Sanavio, F.; Garetto, L.; Severino, A.; Dané, A.; Gammaitoni, L.; Aglietta, M. Differential growth factor requirement of primitive cord blood hematopoietic

stem cell for self-renewal and amplification vs proliferation and differentiation. *Leukemia* **1998**, *12*, 718-727, doi:10.1038/sj.leu.2401003.

23. Tajer, P.; Pike-Overzet, K.; Arias, S.; Havenga, M.; Staal, F.J.T. Ex Vivo Expansion of Hematopoietic Stem Cells for Therapeutic Purposes: Lessons from Development and the Niche. *Cells* **2019**, *8*, 169.

24. Notta, F.; Doulatov, S.; Laurenti, E.; Poeppl, A.; Jurisica, I.; Dick, J.E. Isolation of Single Human Hematopoietic Stem Cells Capable of Long-Term Multilineage Engraftment. *Science* **2011**, *333*, 218-221, doi:doi:10.1126/science.1201219.

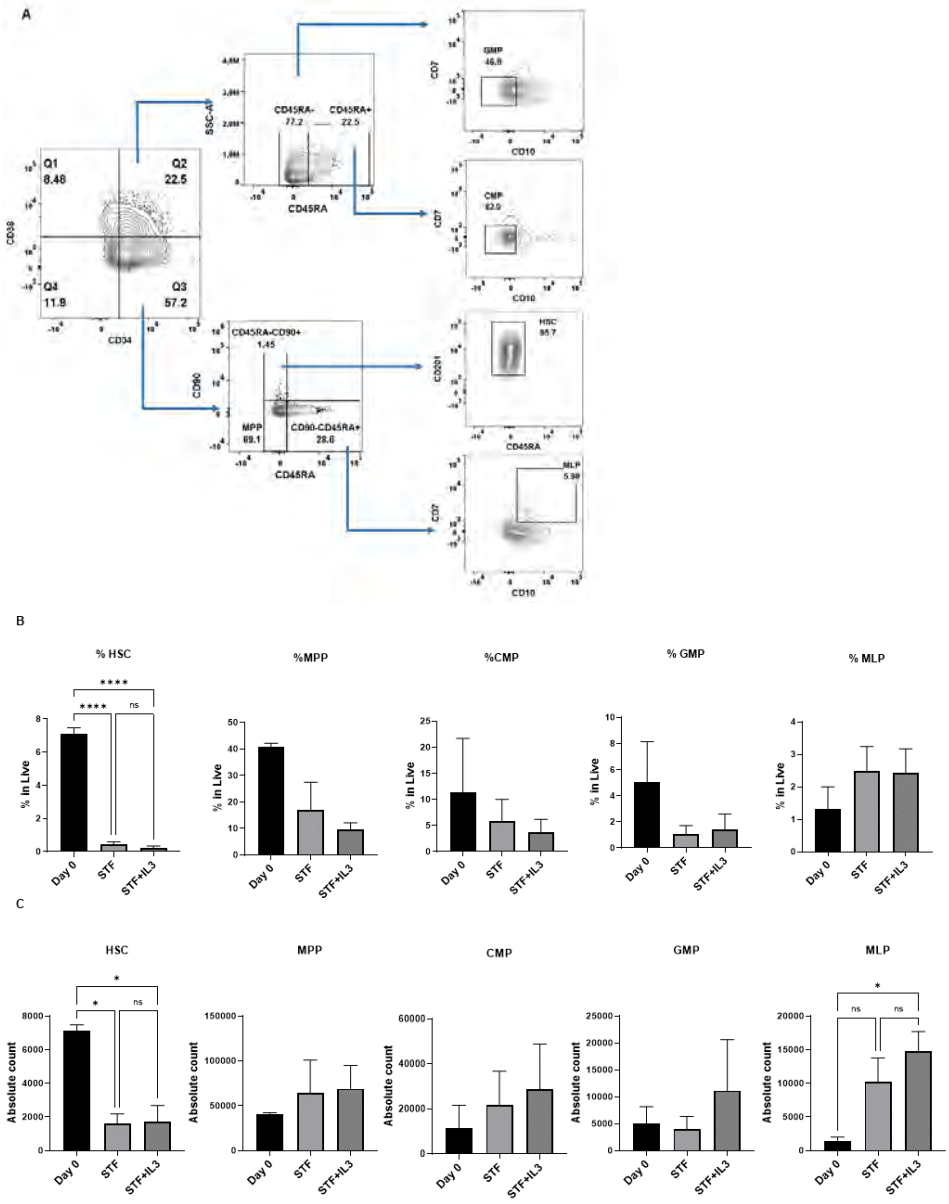
25. Haylock, D.N.; To, L.B.; Dowse, T.L.; Juttner, C.A.; Simmons, P.J. Ex Vivo Expansion and Maturation of Peripheral Blood CD34+ Cells Into the Myeloid Lineage. *Blood* **1992**, *80*, 1405-1412, doi:https://doi.org/10.1182/blood.V80.6.1405.1405.

26. Broxmeyer, H.E.; Hangoc, G.; Cooper, S.; Ribeiro, R.C.; Graves, V.; Yoder, M.; Wagner, J.; Vadhan-Raj, S.; Benninger, L.; Rubinstein, P., et al. Growth characteristics and expansion of human umbilical cord blood and estimation of its potential for transplantation in adults. *Proceedings of the National Academy of Sciences of the United States of America* **1992**, *89*, 4109-4113, doi:10.1073/pnas.89.9.4109.

27. Suda, T.; Suda, J.; Ogawa, M.; Ihle, J.N. Permissive role of interleukin 3 (IL-3) in proliferation and differentiation of multipotential hemopoietic progenitors in culture. **1985**, *124*, 182-190, doi:https://doi.org/10.1002/jcp.1041240203.

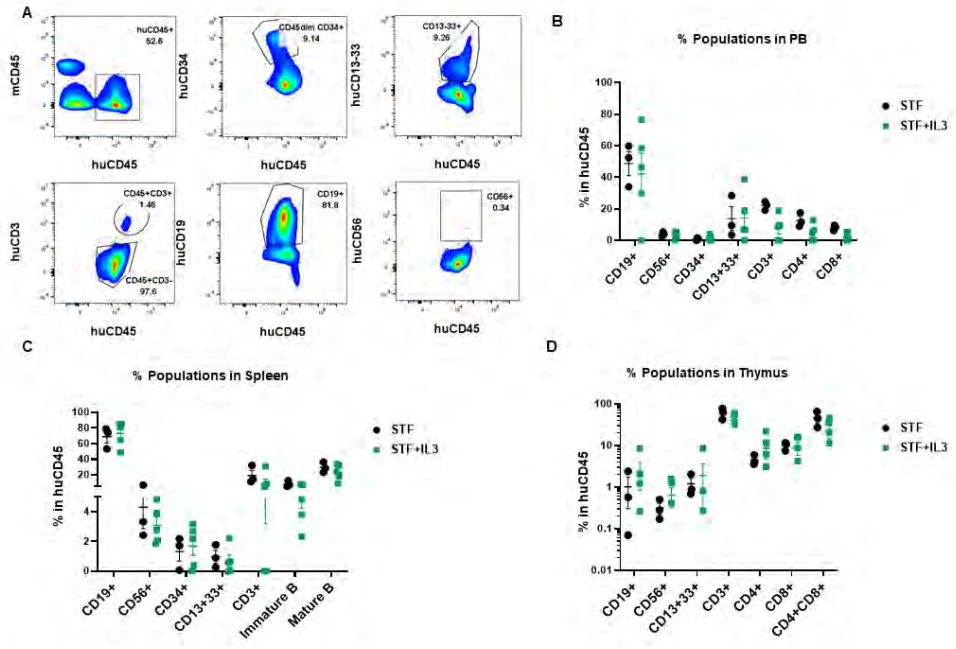
28. Ihle, J.N.; Keller, J.; Oroszlan, S.; Henderson, L.E.; Copeland, T.D.; Fitch, F.; Prystowsky, M.B.; Goldwasser, E.; Schrader, J.W.; Palaszynski, E., et al. Biologic properties of homogeneous interleukin 3. I. Demonstration of WEHI-3 growth factor activity, mast cell growth factor activity, p cell-stimulating factor activity, colony-stimulating factor activity, and histamine-producing cell-stimulating factor activity. *Journal of immunology (Baltimore, Md. : 1950)* **1983**, *131*, 282-287.

29. Matsunaga, T.; Hirayama, F.; Yonemura, Y.; Murray, R.; Ogawa, M. Negative Regulation by Interleukin-3 (IL-3) of Mouse Early B-Cell Progenitors and Stem Cells in Culture: Transduction of the Negative Signals by βc and βIL-3 Proteins of IL-3 Receptor and Absence of Negative Regulation by Granulocyte-Macrophage Colony-Stimulating Factor. *Blood* **1998**, *92*, 901-907, doi:10.1182/blood.V92.3.901 %J Blood.

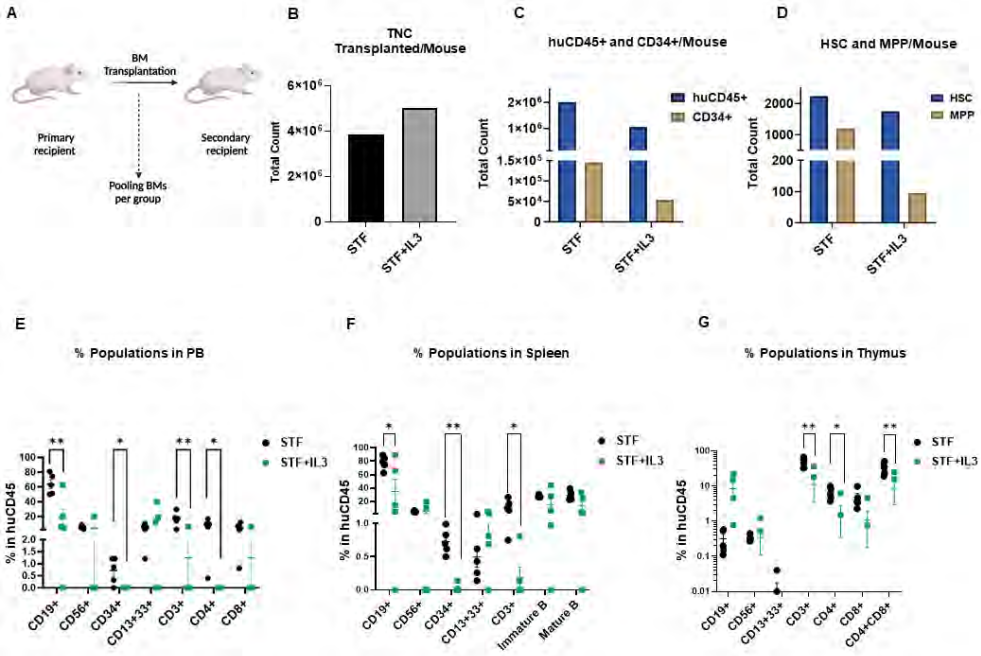


Supplementary Figure S1. Percentages and absolute count of hematopoietic stem cells and progenitors (HSPCs). (A) Representative gating strategy for phenotypic analysis of HSPCs. Hematopoietic stem cell (HSC): $CD34^{+}CD38^{-}CD90^{+}CD45RA^{-}CD201^{+}$, Multipotent Progenitor (MPP): $CD34^{+}CD38^{-}CD45RA^{-}CD90^{-}$, Multi-lymphoid Progenitor (MLP): $CD34^{+}CD38^{-}CD45RA^{+}CD90^{-}CD10^{+}CD7^{+}$, Common Myeloid Progenitor (CMP): $CD34^{+}CD38^{+}CD45RA^{+}CD10^{-}CD7^{-}$, Granulocyte-Macrophage Progenitor (GMP):

$CD34^+CD38^+CD45RA^-CD10^-CD7^-$. (B) Percentage (C) and absolute cell counts of the different progenitor populations at day 0 and day 4 (n=3, *p<0,05, **p<0,01 and ***p<0,001, one-way anova).



Supplementary Figure S2. Human engraftment in organs from primary transplanted NSG mice. (A) Representative of flow cytometry analysis strategy in bone marrow at week 20 post transplantation of different populations within $huCD45^+$. (B-D) Percentages of different populations in PB, Thymus and Spleen.



Supplementary Figure S3.Human engraftment in organs from secondary transplanted NSG mice. (A) Schematic picture of secondary transplantation. (B) Total nucleated cells (TNC) transplanted per mouse in each group. (C) Total count of huCD45^{+} and CD34^{+} cells that each mouse received per group. (D) Graph shows total count of HSC and MPP each mouse received per group at the time of secondary transplantation. (E-G) Engraftment of different populations in PB, Thymus and Spleen at week 20 post transplantation (n=5; *p<0,05, **p<0,01 and ***p<0,001, unpaired t-test).

Supplementary Table S1: List of antibodies used.

Name	Fluorochrome	Clone	Source
hCD1a	APC	HI149	BD Biosciences
hCD3	BV786	SK7	BD Biosciences
hCD4	BUV805	SK3	BD Biosciences
hCD5	BV480	UCHT2	BD Biosciences
hCD7	Pe-Cy5	CD7-6B7	Biolegend
hCD8	BV650	RPA-T8	Biolegend
hCD8	BUV496	RPA-T8	BD Biosciences
hCD10	APC-Cy7	HI10a	Biolegend
hCD13	Percp-Cy5.5	WM15	BD Biosciences
hCD19	PE-Cy7	HIB19	Invitrogen
hCD19	BV421	HIB19	Biolegend
hCD20	BV570	2H7	Biolegend
hCD33	Percp-Cy5.5	WM53	BD Biosciences
hCD34	Pe-CF594	581	BD Biosciences
hCD34	BV650	561	Biolegend
hCD38	Pe-Cy7	HIT2	Biolegend
hCD41	BV421	HIP8	Biolegend
hCD45	V450	HI30	BD Biosciences
hCD45RA	BV711	HI100	Biolegend
hCD45RA	BV510	HI100	BD Biosciences

hCD49f	PerCP-ef710	ebio-GoH3	Invitrogen
hCD56	PE-Cy5	B159	BD Biosciences
hCD56	APC-Cy7	HCD-56	Biolegend
hCD62L	BV605	DREG-56	BD Biosciences
hCD71	AF700	M-A712	BD Biosciences
hCD90	APC	5 e10	Biolegend
hCD201	PE	RCR-401	Biolegend
hIgM	BV650	MHM-88	BD Biosciences
hIgD	BV480	IA6-2	BD Biosciences
hTCRgd	BV510	11F2	BD Biosciences
hTCRab	PE	MHM-88	BD Biosciences
mCD45	FITC	30-F11	BD Biosciences
Zombie NIR	NIR	-	Biolegend