Hematopoietic stem cell (HSC) transplantation is a routinely used therapeutic method. Most of all currently registered stem cell-related clinical trials focus on HSCs. Selecting the most potent HSC population might improve the beneficial stem cell related therapeutic effect.

To analyze c-kit+ subpopulations for their proliferation and differentiation capacity, murine bone marrow c-kit+ cells were sorted for lin−, CD34, Sca1 and CD45. Proliferation potential was analyzed using a Colony-Forming-Unit (CFU) assay. Surface markers and gene expression were characterized by flow cytometry and real-time PCR.

Prior to CFU assay entire cell population was viable, lin and c-kit+. Their purity exceeded 92%. The major subpopulation was CD45+CD34+Sca1− (21.25 ± 4.25%), followed by CD45+CD34+Sca1+ (15.00 ± 4.23%). Cells positive for CD45, CD34 and Sca1 and cells negative for all three markers formed smaller populations (0.74 ± 0.40% and 1.03 ± 1.25%). After CFU assay we found significant differences in the CFU count of analyzed subpopulations (P≤0.0026). CD45+CD34+Sca1+ cells displayed the highest CFU count per utilized cell (0.470 ± 0.055; n=3), followed by CD45+CD34+Sca1− cells (0.162 ± 0.019; n=5). CD45+CD34+Sca1− and CD45+CD34+Sca1+ cells showed low CFU frequency (0.0032 ± 0.0009 and 0.0035 ± 0.0027, n=5, difference n.s.; Figure 1).

Interestingly, original CD45+CD34+Sca1− cells after CFU displayed a larger Sca1+ population than CD45+CD34+Sca1+ cells (14.51 ± 5.21% versus 3.84 ± 0.72% CD34+Sca1−c-kit+lin− CD45+ cells). In CD45+CD34+Sca1−-derived cells, SPP1 and GATA2 genes, which are present in HSC niche, were upregulated whereas Wnt3a and VEGFA genes, which are mainly expressed in active and differentiating HSC, were strongly downregulated compared to cells derived from the CD45+CD34+Sca1+ subpopulation.

In conclusion, we present viable lin− c-kit+ cell subpopulations, specified by differential CD45, CD34 and Sca1 protein surface expression, are highly heterogeneous in proliferation and differentiation capacity. CD45+CD34+Sca1+ cells proliferate more readily and may differentiate more distinctively than CD45+CD34+Sca1−, while CD34+Sca1− cells display a low proliferation potential. These findings might help deepen
the knowledge in c-kit^ HSC plasticity and therapeutic potential.

Figure 1