The majority of the *in vitro* erythroid expansion potential resides in CD34$^-$ cells, outweighing the contribution of CD34$^+$ cells and significantly increasing the erythroblast yield from peripheral blood samples

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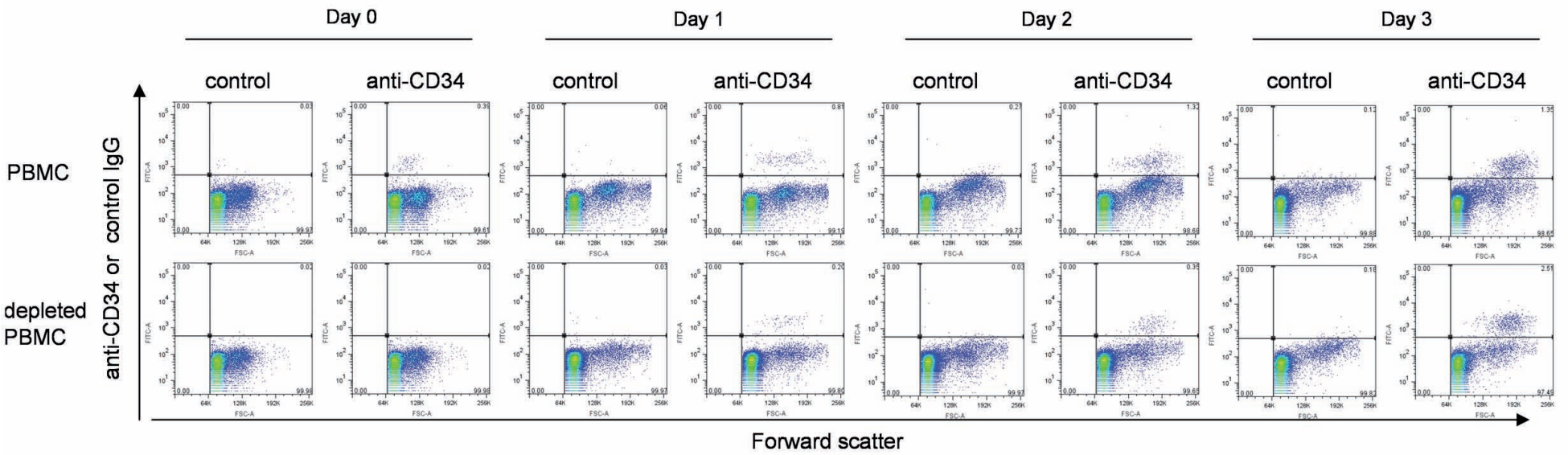
Citation: van den Akker E, Satchwell TJ, Pellegrin S, Daniels G, and Toye AM. The majority of the in vitro *erythroid expansion potential resides in CD34$^-$ cells, outweighing the contribution of CD34$^+$ cells and significantly increasing the erythroblast yield from peripheral blood samples. Haematologica 2010;95(9): 1594-1598. doi:10.3324/haematol.2009.019828*

Online Supplementary Table S1. Erythroid colony formation capacity during the first four days of *in vitro* culture. Erythroid colony formation at day 0, 2 and 4 of *in vitro* expansion. 0.5 million total PBMC or PBMC depleted for CD34$^+$ cells were plated into semi-solid methylcellulose medium at isolation from peripheral blood (day 0) or after two and four days of *in vitro* culture, as described in *Design and Methods*. To allow the specific outgrowth of erythroid colonies, the methylcellulose media (Stem Cell Technologies) was supplemented with SCF (100ng/mL), Epo (2U/mL) and IL-3 (ng/mL). Colony forming units (CFUs) of erythroid nature were counted after 14 days and are presented as an average. The number of independent experiments are indicated (SD= standard deviation between experiments).

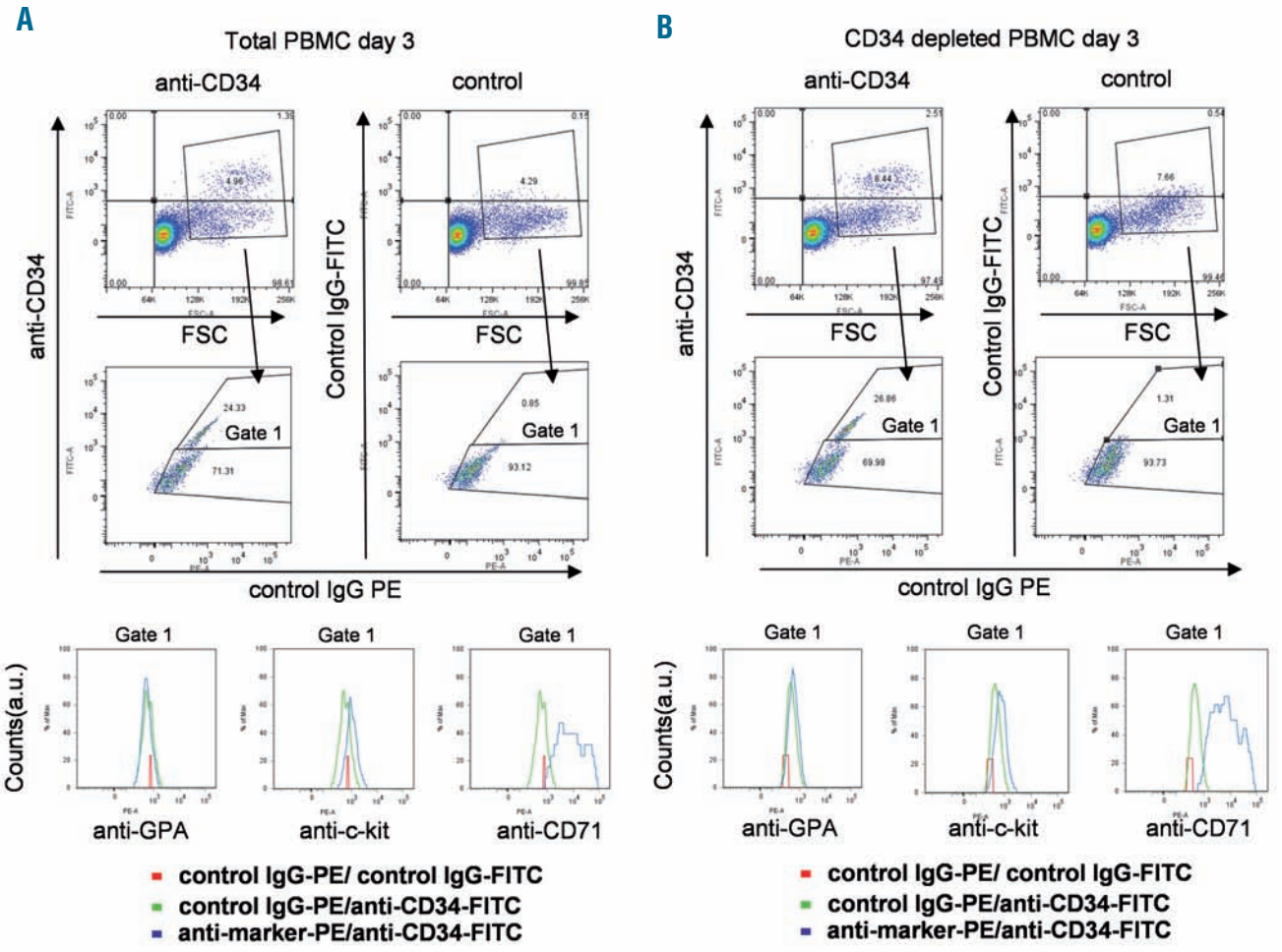
	Total PBMCs		CD34- PBMCs	
Days of *in vitro* culture	Erythroid CFU's	SD/N exp	Erythroid CFU's	SD/N exp
0	91	15 N=4	4	4 n=4
2	229	9 N=4	131	NA n=2
4	2039	263 N=4	613	NA n=2

Input = 0.5×10^6 cells/experiment



Online Supplementary Figure S1. PBMC depleted of CD34⁺ cells become CD34⁺ during expansion. Dot plots showing control IgG or anti-CD34 staining against forward scatter (FSC) for total PBMC cultures (top panels) and CD34⁺ cell depleted PBMC cultures (bottom panels). Note that in the bottom panel second dot plot, the CD34⁺ cells are totally depleted but become CD34⁺ during the next days (subsequent panels). The figure shows one representative experiment out of four independent experiments.

Online Supplementary Figure S2. CD34$^+$ cells at day 3 of expansion are c-kit$^+$/CD71$^+$/GPA$^-$. CD34$^+$ cells at day 3 of expansion are c-kit$^+$/CD71$^+$/GPA$^-$. PBMC depleted for CD34$^+$ cells (lower panels) or not (upper panels) were expanded as indicated in *Design and Methods*. The dot plots delineate the specific population used in the histograms below namely the CD34$^+$ (FITC$^+$) population with medium to high forward scatter (the low forward scatter population are lymphocytes). The histograms show the expression of GPA (BRIC256-PE), c-kit (CD117-PE) and CD71 (CD71-PE) within the CD34$^+$/FSC$_{med/high}$ population at day 3 in total PBMC (A) and CD34 depleted PBMC (B). Note that in both (A) and (B), the CD34$^+$/FSC$_{high}$ population is c-kit$^+$/CD71$^+$ but GPA negative and thus are termed as common megakaryocytic/erythroid progenitors as the earlier common lymphoid progenitor is CD71 low to negative but CD34$^+$/c-kit$^+$/GPA$^-$. The figures here are representative of four independent experiments.



Online Supplementary Figure S3. Cells after the second Percoll on day 5 are c-kit$^+$/CD71$^+$/GPA$^-$/CD34$^-$ and thus identified as early erythroid progenitors. Cells after the second Percoll on day 5 are c-kit$^+$/CD71$^+$/GPA$^-$/CD34$^-$ and thus identified as erythroid progenitors. The transition of the common megakaryocyte/erythroid progenitor to the erythroid progenitor is accompanied with the loss of CD34 and a further increase in CD71 and c-kit expression. The dot plots show control-FITC (upper panel) and anti-CD34-FITC (lower panel) staining against PE-positiveness (the specific antibodies conjugated to PE staining are indicated below the dot plot). The insets in the dot plots in the upper panel show the respective PE-histograms of the dot plots in order to compare with the histograms at day 3 in Online Supplementary Figure S2. Note the increase in CD71 and c-kit expression compared to day 3 and the absence of GPA. Furthermore, a low expression of CD34 remains on approximately 13% of the cells. Combined, the data show that 87% of the cells are CD34$^-$/GPA$^-$/c-kit$^+$/CD71$_{high}$. The figures are representative of three independent experiments.