

CD34⁺/CD38⁻ stem cells in chronic myeloid leukemia express Siglec-3 (CD33) and are responsive to the CD33-targeting drug gemtuzumab/ozogamicin

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Online Supplementary Table S1. Specification of monoclonal antibodies (mAb).

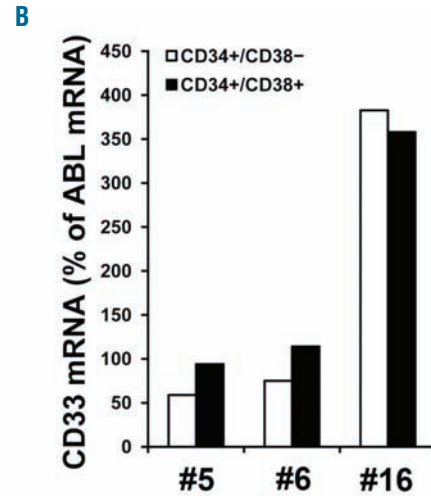
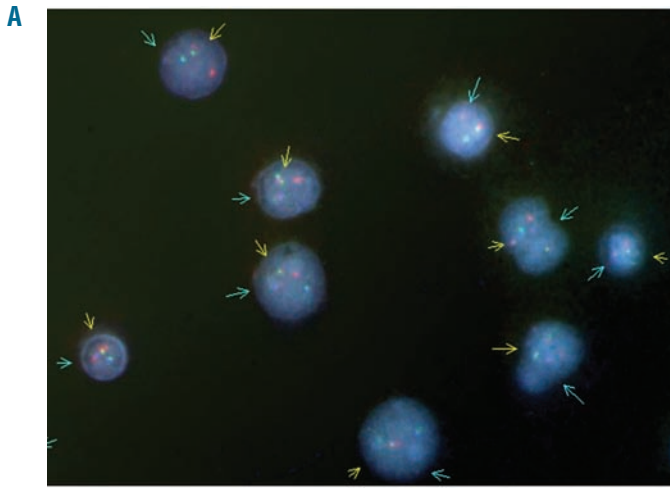
Antigen	Clone	Fluorochrome	Isotype	Manufacturer
Isotype control	MOPC-21	PE	mouse IgG1	BD Biosciences
CD33	WM53	PE	mouse IgG1	BD Biosciences
CD34	581	FITC and PE	mouse IgG1	BD Biosciences
CD38	HIT2	APC	mouse IgG1	BD Biosciences
CD45	2D1	PerCP	mouse IgG1	BD Biosciences
MDR1/CD243	15D3	PE	mouse IgG1	BD Biosciences
IL-3R α /CD123	32703	PE	mouse IgG1	R&D Systems

PE: phycoerythrin; FITC: fluorescein isothiocyanate; APC: allophycocyanin; PerCP: peridinin chlorophyll protein; BD: Becton Dickinson.

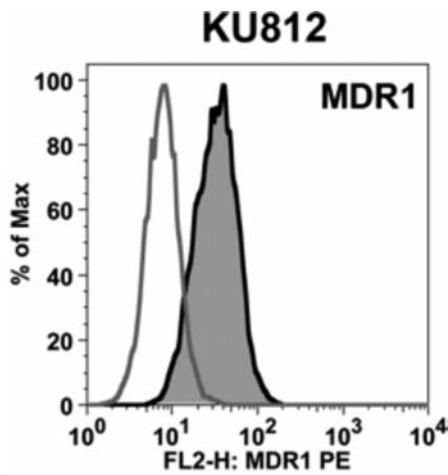
Online Supplementary Table S2. Antibody combinations used in flow cytometry experiments.

Tube #	FITC	PE	PerCP	APC
1	blank	blank	blank	blank
2	CD34	mouse IgG1	CD45	CD38
3	CD34	CD33	CD45	CD38
4	CD34	CD243	CD45	CD38
5	CD34	CD123	CD45	CD38

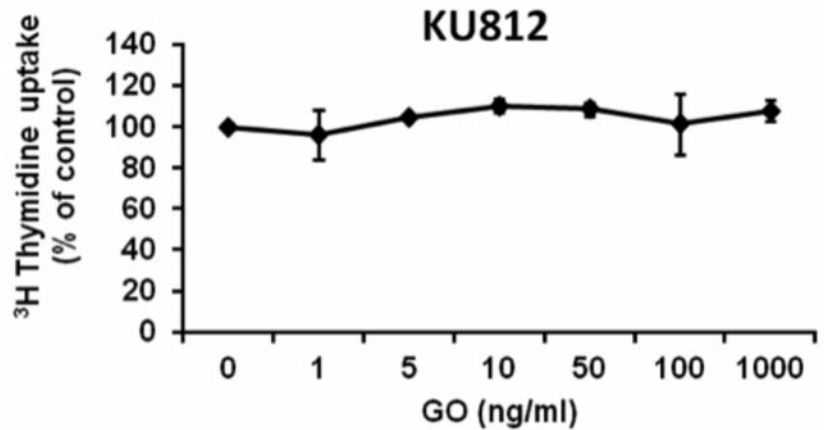
FITC: fluorescein isothiocyanate; PE: phycoerythrin; PerCP: peridinin chlorophyll protein; APC: allophycocyanin.



Online Supplementary Figure S1. Detection of BCR/ABL and CD33 mRNA in highly enriched CML stem cells. (A) Fluorescence *in situ* hybridization (FISH) of purified leukemic stem cells. FISH was performed on cytospin-preparations of CML cells using a triple color-staining approach with one probe specific for ABL1 (red) and two (green and blue) specific for BCR. Red-green (yellow arrows) and red-blue (blue arrows) fusion signals represent BCR/ABL fusion spots and thus the t(9;22). (B) Detection of CD33 mRNA in purified CD34⁺ leukemic progenitor cells by qPCR. CD34⁺/CD38⁻ cells (white bars) and CD34⁺/CD38⁺ cells (black bars) were isolated in 3 patients (#5, #6, and #16; Table 1) and examined for CD33 mRNA expression as described in the text. CD33 transcript levels are expressed as percentage of ABL mRNA levels.



Online Supplementary Figure S2. Detection of MDR1 in the CML cell line KU812 by flow cytometry. The CML cell line KU812 was analyzed for expression of MDR1 (black filled histogram) by the monoclonal antibody 15D3. The isotype control is also shown (gray white histogram).



Online Supplementary Figure S3. Effects of gemtuzumab/ozogamicin (GO) on growth of KU812 cells. KU812 cells were cultured in the presence or absence (0) of GO (1-1,000 ng/mL) at 37 °C for 48 h. After incubation, ³H-thymidine uptake was measured. Results are expressed as percent of control and represent the mean ± S.D. of triplicates.