Comparative analysis of proliferative potential and clonogenicity of MACS-immunomagnetic isolated CD34⁺ and CD133⁺ blood stem cells derived from a single donor

D. Freund^{*,†}, J. Oswald^{*}, S. Feldmann^{*}, G. Ehninger^{*}, D. Corbeil[†]
and M. Bornhäuser^{*}

^{*}Medical Clinic and Polyclinic I, University Hospital, and

[†]Tissue Engineering Laboratories, BIOTEC, Technical University of Dresden, Dresden, Germany

Received 14 March 2006; revision accepted 7 April 2006

Abstract. A novel stem cell marker prominin-1 (CD133) has been shown to be expressed on a subpopulation of CD34⁺ haematopoietic stem and progenitor cells. The aim of this study was to compare in parallel commercially available CD34⁺ and CD133⁺ isolation methods based on paramagnetic bead-coupled antibodies using clinical-grade samples of mobilized peripheral blood from 10 individual healthy donors under identical conditions. The CD133 negative fraction from the first selection was used for CD34⁺ enrichment to obtain an additional CD34⁺/CD133⁻ population. Although no significant difference in total cell expansion between cells isolated from the three procedures was observed in a 7-day cytokine-driven suspension culture, the long-term culture-initiating cell assay demonstrated that cells derived by CD34⁺ isolation contain less primitive progenitors than those isolated based on CD133⁺ selection. Interestingly, CD34⁺-enriched progenitors, especially the CD34⁺/CD133⁻ fraction, contained a significantly higher proportion of erythroid colony-forming cells, whereas the highest content of myeloid colony-forming cells was concentrated in the CD133⁺ selected cells. These subtle differences between CD34⁺ and CD133⁺ immunomagnetic selection will have to be explored for their potential clinical relevance.

INTRODUCTION

Haematopoiesis is maintained by a small number of haematopoietic stem and progenitor cells (HSCs/HPCs), which are able to proliferate (self-renew) and to differentiate into all lineages of the haematopoietic system. Throughout the last decade, monoclonal antibodies against the glycoprotein CD34 (Krause *et al.* 1996) have been used to identify and isolate HSCs/HPCs. Initially, after using immunoadsorptive methods and fluorescence-activated cell sorting, the selection of CD34⁺ cells by paramagnetic bead-coupled antibodies became the method of choice in clinical stem cell transplantation (Beaujean 1997).

Correspondence: Martin Bornhäuser, Medizinische Klinik und Poliklinik I, University Hospital, Fetscherstrasse 74, 01307 Dresden, Germany. Tel.: +49 351 458 4704; Fax: +49 351 458 5362; E-mail: martin.bornhaeuser@uniklinikum-dresden.de
Present address: J. Oswald, Forschungszentrum Rossendorf, Bautzner Landstraße 128, D-01328 Dresden, Germany.

In the meantime, a new cell surface antigen initially called AC133 antigen (Yin *et al.* 1997) and now referred to as prominin-1 or CD133 (Weigmann *et al.* 1997; Bühring *et al.* 2002; Fargeas *et al.* 2003, 2006) has been demonstrated to be expressed on a subpopulation of CD34⁺ HSCs/HPCs (Miraglia *et al.* 1997; Yin *et al.* 1997; Corbeil *et al.* 2000). A unique population of CD34-negative HSCs/HPCs expressing CD133 with primitive stem cell properties has also been reported (Gallacher *et al.* 2000; Kuci *et al.* 2003). The CD133⁺ HSCs/HPCs have the capacity to reconstitute the entire immune system of lethally irradiated mice (de Wynter *et al.* 1998), and besides their haematopoietic capacity it has been shown that CD133⁺ cells are capable of *in vitro* differentiation into neuronal and endothelial cells (Gehling *et al.* 2000; Quirici *et al.* 2001) as well as into myoblasts (Torrente *et al.* 2004), underlining a much higher plasticity of CD133⁺ cells compared to CD34⁺ cells.

A large-scale immunomagnetic selection of CD133⁺ cells allowed enriching a sufficient number of HSCs/HPCs to perform HSC transplantation (Gordon *et al.* 2003), and clinical trials with leukaemic children have proven the feasibility of CD133⁺ cells for allogeneic transplantation (Koehl *et al.* 2002; Lang *et al.* 2004). Recently, we could show a rapid establishment of donor dendritic cell chimerism after allogeneic transplantation of CD133⁺ selected cells (Bornhäuser *et al.* 2005). The immunomagnetic isolation procedure of HSCs/HPCs based on CD133 appears thus to be an interesting alternative to CD34. Does CD133⁺ selection offer any relevant advantage to available CD34⁺ selection techniques? Several groups have investigated this particular issue (de Wynter *et al.* 1998; Goussetis *et al.* 2000; Matsumoto *et al.* 2000; Pasino *et al.* 2000; Kobari *et al.* 2001; Dimitriou *et al.* 2003), however, the outcome of these studies varied considerably. Discrepancies may have arisen from differences in the haematopoietic cell sources, but as discussed by Matsumoto *et al.* (2000), they may also be attributed to the methods of cell sorting or the clonogenic assay used. In this report we decided to re-investigate the properties of mobilized peripheral blood stem cells after enrichment with either anti-CD34 or anti-CD133 paramagnetic beads. Because the cellular variability between each individual might also be high, we used the different selection antigens in parallel on HSCs/HPCs derived from the same donor under identical conditions.

MATERIALS AND METHODS

Haematopoietic stem/progenitor cell enrichment

Mobilized peripheral blood from healthy donors was collected after informed consent and approval by the local ethics committee. Mobilization was achieved by subcutaneous injection of 7.5 µg/kg/day granulocyte colony-stimulating factor (G-CSF; Granocyte, Chugai Pharma, Frankfurt, Germany) on five consecutive days. The leukapheresis product derived from a single donor was divided into two aliquots, and HSCs/HPCs were isolated immediately by immunomagnetic separation using either CD34 or CD133 selection kits according to the manufacturer's protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). The magnetically labelled CD34⁺ or CD133⁺ cells recovered from the first column were run over a second column in order to obtain higher purity. Cells in the first flow-through of the CD133 selection were collected and subjected to a CD34 selection, leading to an enrichment of CD34⁺CD133⁻ cells (referred to as CD133-FT; see Fig. 1a_{iii}).

Flow cytometry

Isolated as well as expanded HSCs were labelled with fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)- and allophycocyanin (APC)-conjugated monoclonal antibodies. Then

Figure 1. Isolation and characterization of HSCs/HPCs derived from a single donor and their multilineage differentiation capacity. (a) Illustration of the experimental setup. The leukapheresis product derived from single donors ($n = 10$) was divided into two aliquots, and HSCs/HPCs were enriched immediately using either CD34⁺ or CD133⁺ coupled paramagnetic MACS® MicroBeads. The magnetically labelled CD34⁺ or CD133⁺ cells recovered from the first column were run over a second column (2X) in order to obtain a higher purity. Cells in the first flow-through of the CD133 selection were collected and subjected to a CD34 selection, leading to an enrichment of CD34⁺CD133⁺ cells (CD133-FT). Expression of CD34 and CD133 were evaluated by FACS and one representative experiment is shown (panels i–iii). (b) The clonogenicity of CD34-MACS, CD133-MACS and CD133-FT selected cells was tested by a methylcellulose assay. Mean and standard error of the mean are shown. Asterisks indicate a significant difference compared to CD133-FT, as calculated by two-tailed Student's *t*-test ($P < 0.05$). All experiments were carried out in triplicate.

200 μ l of the cell suspension (2×10^5) were incubated with 10 μ l of the antibody for 30 min at 4 °C. After washing with PBS, 30 000 events were acquired on a FACSCalibur (BD Biosciences, Heidelberg, Germany) equipped with 488 and 635 nm lasers. Instrument settings and gating strategies were established using appropriate controls, and data were analysed using CELLQUEST PRO software (BD Biosciences). The following antibodies were used: CD34-APC (clone 581), CD38-FITC (clone HIT2), CD45-FITC (HI30), CD184-PE (clone 12G5; all purchased from BD Biosciences) and CD133-PE (AC133-1; Miltenyi Biotec).

Proliferation assay of MACS-enriched cells

Magnetic assisted cell sorting (MACS)-enriched cells (1×10^4 /ml) were cultured for 7 days in six-well plates containing 3 ml HSC medium at 37 °C in a humidified 5% CO₂ atmosphere. The HSC medium consisted of serum-free medium (CellGro SCGM, CellGenix, Freiburg, Germany) containing 150 ng/ml stem cell factor (SCF; CellSystems, St. Katharinen, Germany), 150 ng/ml foetal liver tyrosine kinase-3 ligand (FLT3-L), and 50 ng/ml interleukin 3 (IL-3) (both R&D Systems, Mannheim, Germany).

Colony-forming unit and long-term culture – initiating cell assays

Multilineage differentiation capacity of the MACS-enriched HSCs/HPCs was evaluated using a methylcellulose-based colony assay. Briefly, 1×10^3 cells were vigorously mixed with 1 ml complete methylcellulose medium (MethoCult H4435; StemCell Technologies, Vancouver, Canada). Aliquots of 330 μ l were plated in triplicates into 24-well plates and were cultivated at 37 °C under a humidified 5% CO₂ atmosphere. After 14 days each well was scored for the number of burst-forming unit – erythroid (BFU-E), colony-forming unit – granulocyte macrophage (CFU-GM), CFU – macrophage (CFU-M) and CFU – granulocyte, erythrocyte, macrophage, megakaryocyte (CFU-GEMM) according to the standard criteria (Coutinho *et al.* 1993).

The frequency of long-term culture – initiating cell (LTC-IC) at 5 weeks was determined using a limiting dilution assay as previously described (Matsumoto *et al.* 2000). Briefly, monolayers of M2-10B4 cells were established in 96-well plates. One day before the addition of the HSCs/HPCs, the feeder cells were irradiated at 80 Gy. HSCs/HPCs were resuspended in LTC medium (MyeloCult H5100; StemCell Technologies; containing 10^{-6} M hydrocortisone) at three different concentrations. Aliquots of 100 μ l were then added with 12 replicates per dilution to the feeder layer and maintained at 37 °C under a humidified 5% CO₂ atmosphere. One-half of the medium was changed every week. After 5 weeks LTC medium was replaced by 100 μ l of methylcellulose medium (see above), and cells were cultured for additional 14 days. At the end, all wells were scored for the presence or absence of colonies and LTC-IC frequencies were calculated.

Statistical analysis

Results from all experiments are expressed as mean $\pm$ standard error of the mean (SEM). Data were further analysed by two-tailed paired Student's *t*-test. Probability values < 0.05 designated significant differences between test points. All analyses were performed using SPSS 12.0 for Windows (SPSS Inc., Chicago).

RESULTS AND DISCUSSION

The HSCs/HPCs were enriched from G-CSF mobilized peripheral blood derived from individual donors ($n = 10$) using CD34- and CD133-coupled paramagnetic beads in parallel under identical

Table 1. Comparison of expression of CD34 and CD133, expansion rate and expansion of CD34⁺ cells for the different isolation procedures

	CD34-MACS		CD133-MACS		CD133-FT	
	Before culture	After culture	Before culture	After culture	Before culture	After culture
% CD34 ⁺	94.0 ± 2.1*	34.6 ± 3.8*	97.1 ± 0.8*	37.8 ± 3.7*	90.3 ± 2.5	24.7 ± 3.9
% CD133 ⁺	75.1 ± 3.7*	7.3 ± 1.8	92.6 ± 1.8*	9.8 ± 1.7	21.5 ± 7.3	5.8 ± 1.5
Total cell expansion	n.a.	48.4 ± 7.3-fold	n.a.	52.6 ± 6.0-fold	n.a.	41.7 ± 8.4-fold
CD34 ⁺ cell expansion	n.a.	19.6 ± 3.9-fold*	n.a.	19.4 ± 3.1-fold*	n.a.	9.7 ± 2.1-fold

G-CSF mobilized peripheral blood from a single donor ($n = 10$) was divided into two aliquots and subjected to MACS enrichment using either anti-CD34 or anti-CD133 paramagnetic beads. Expression of CD34 and CD133 was evaluated by FACS before and after 1 week of *in vitro* culture under serum-free conditions. Total expansion and expansion of CD34⁺ cells were determined. Mean and standard error of the mean are shown. Asterisks indicate a significant difference compared to CD133-FT, as calculated by two-tailed paired Student's *t*-test ($P < 0.05$). n.a., not applicable.

conditions. Flow cytometry analyses revealed that $74.9 \pm 3.7\%$ of the HSCs/HPCs derived from CD34-MACS selection to be CD34⁺CD133⁺, whereas $19.3 \pm 2.2\%$ of them exhibited a CD34⁺CD133⁻ phenotype (Fig. 1a, panel i), this is consistent with previous results (Yin *et al.* 1997; Pasino *et al.* 2000). The CD133-MACS-selected cells contained more than $92.5 \pm 1.8\%$ HSCs/HPCs with the CD34⁺CD133⁺ phenotype and $4.7 \pm 1.2\%$ of the cells were CD34⁺CD133⁻ (Fig. 1a, panel ii). Under these conditions, the viability of isolated cells was greater than 95%. Subjecting unbound cells from flow-through of the CD133 selection to an immunomagnetic isolation by anti-CD34 led to an enrichment of CD34⁺CD133⁻ HSCs/HPCs (referred to as CD133-FT), which represents $70.4 \pm 7.7\%$ of total cells (Fig. 1a, panel iii). This double selection, however, decreased the viability of isolated cells down $\approx 90\%$. Even after two enrichment columns there were still some residual CD133⁺ cells left ($21.5 \pm 7.3\%$), which might represent CD133^{dim} cells incapable of strong binding to the beads. Representative experiments showing these three distinct cell populations are illustrated in Fig. 1a (see panels i–iii). Irrespective of the separation procedure, approximately 90% and 60% of the HSCs/HPCs exhibited CD34⁺CD38⁺ and CD34⁺CD184⁺ phenotypes, respectively.

We investigated the expansion capacities of CD34-MACS and CD133-MACS selected cells as well as CD133-FT cells. *Ex vivo* expansion of the three enriched cell populations was carried out for 7 days under serum-free conditions in the presence of a combination of early acting cytokines (SCF, 150 ng/ml; FLT3-L, 150 ng/ml; IL-3, 50 ng/ml). As summarized in Table 1, no significant difference in total cell expansion between cells isolated from the three procedures was observed. However, a significant reduction of CD34⁺ cells was detected in the extended product derived from CD133-FT compared to those derived from CD34-MACS and CD133-MACS (Table 1). These data indicate that HSCs/HPCs enriched from CD34⁻ or CD133-based paramagnetic microbead selection have a similar propensity to expand and to maintain a significant proportion of CD34⁺ cells, whereas HSCs/HPCs derived from CD133-FT have lost the property to maintain a primitive phenotype. This phenomenon might be explained, at least in part, by the fact that CD34⁺CD133⁻ cells, which represented the major cell fraction of the CD133-FT population, were more committed than CD34⁺CD133⁺ cells. de Wynter and colleagues (1998) have indeed previously demonstrated that CD34⁺CD133⁻ HSCs/HPCs have a lower LTC-IC capacity compared to CD34⁺CD133⁺ HSCs/HPCs.

Next, we evaluated the multilineage differentiation capacity of the MACS-enriched HSCs/HPCs using a methylcellulose-based colony assay. Although this did not reveal major differences in total colony numbers (Fig. 1b), the relation of BFU-E to CFU-GM was changed between HSCs/HPCs derived from CD34-MACS and CD133-MACS from 2 : 3 to 1 : 3, respectively (Fig. 1b). These observations suggest that cells harbouring the CD34⁺CD133⁻ phenotype, which represents $\approx$ 20% of CD34-MACS isolated cells and less than 5% in CD133-MACS, have a greater propensity to generate BFU-E. This assumption is confirmed by the analysis of CD133-FT, where the relation BFU-E to CFU-GM was mainly inverted to a ratio of 3 : 1 (Fig. 1b). The numbers of CFU-M and CFU-GEMM were comparable throughout the different isolation procedures (Fig. 1b). We found comparable ratios of determined progenitors (CFU-GM and BFU-E) in the 7-day *in vitro* expanded cell fractions, although the overall frequency of clonogenic cells was reduced (data not shown). Finally, LTC-IC assays revealed a frequency of $1/175 \pm 63$ vs. $1/383 \pm 120$ for CD133-MACS and CD34-MACS, respectively. There were practically no primitive haematopoietic cells present in the CD133-FT fraction, displayed by a LTC-IC frequency of $1/>10.000$. These data are consistent with results obtained for steady-state peripheral blood (Matsumoto *et al.* 2000).

Taken together, our experiments performed with HSCs/HPCs derived from 10 individual donors and immunoisolated simultaneously by two distinct, clinically used, cell surface antigens, CD34 or CD133, revealed no difference in terms of expansion properties. However, our CFU and LTC-IC assays show that HSCs/HPCs derived from the CD34 isolation protocol were enriched in erythroid precursors and contained less primitive haematopoietic cells, which might be attributed to the presence of a higher number of CD34⁺CD133⁻ cells. Similar conclusions have been reported, although the HSC/HPCs were isolated from other sources than mobilized peripheral blood, from various donors and using alternative methods such as FACS sorting (Yin *et al.* 1997; de Wynter *et al.* 1998; Gallacher *et al.* 2000; Matsumoto *et al.* 2000; Gordon *et al.* 2003). The larger number of CFU-GM generated from HSCs/HPCs enriched from the CD133 isolation protocol is in line with recent observations from a phase I trial, where increased numbers of dendritic cells were found in patients after stem cell transplantation with CD133-selected cells (Bornhäuser *et al.* 2005) as well as the greater capacity of CD34⁺CD133⁺ cells to produce dendritic cells *in vitro* (de Wynter *et al.* 1998).

Clinical studies suggest no significant difference so far in terms of engraftment or other relevant clinical end-points using either CD133⁺ or CD34⁺ selected cells (Lang *et al.* 2004; Bornhäuser *et al.* 2005), but still the differences in content of lineage-committed HPCs might be relevant in certain indications. One could envision the preferential use of CD34⁺ selected cells in patients in whom fast erythroid engraftment is of special importance, whereas the use of CD133⁺ selected cells could be advantageous when selected cells are subsequently cultured *in vitro*. In addition, gene transduction protocols might be more efficient when CD133⁺ cells are used to target the myeloid and macrophage lineages. Pre-clinical studies with biological surrogate markers will have to be performed to confirm these hypotheses.

ACKNOWLEDGEMENTS

We thank Kristina Hölig, Verona Schwarze, Anja Maiwald, Anja Weisske and Diana Döhler for providing the clinical leukapheresis samples. This work was supported by the MeDDrive program of the Medical Faculty of the Technical University Dresden. D.C. was supported by the Deutsche Forschungsgemeinschaft (SPP 1109, CO 298/2-1 and CO 298/2-2).

REFERENCES

- Beaujean F (1997) Methods of CD34⁺ cell separation: comparative analysis. *Transfus. Sci.* **18**, 251–261.
- Bornhäuser M, Eger L, Oelschlaegel U, Auffermann-Gretzinger S, Kiani A, Schetelig J, Illmer T, Schaich M, Corbeil D, Thiede C, Ehninger G (2005) Rapid reconstitution of dendritic cells after allogeneic transplantation of CD133⁺ selected hematopoietic stem cells. *Leukemia* **19**, 161–165.
- Bühning HJ, Marxer A, Lammers R, Wissinger B (2002) New CD antigens. CD133 Cluster Report. *Leucocyte Typing*, Mason D (ed.). Oxford University Press, Oxford, UK, pp. 622–623.
- Corbeil D, Röper K, Hellwig A, Tavian M, Miraglia S, Watt SM, Simmons PJ, Peault B, Buck DW, Huttner WB (2000) The human AC133 hematopoietic stem cell antigen is also expressed in epithelial cells and targeted to plasma membrane protrusions. *J. Biol. Chem.* **275**, 5512–5520.
- Coutinho LH, Gillece MH, de Wynter EA, Will A, Testa NG (1993) Clonal and long-term cultures using human bone marrow. In: Testa NG, Molineux G, eds. *Hemopoiesis: a Practical Approach*. Oxford University Press, Oxford, UK, pp. 75–106.
- Dimitriou H, Vorgia P, Stiakaki E, Mavroudis D, Markaki EA, Koumantakis E, Kalmanti M (2003) *In vitro* proliferative and differentiating characteristics of CD133(+) and CD34(+) cord blood cells in the presence of thrombopoietin (TPO) or erythropoietin (EPO). *Leuk. Res.* **27**, 1143–1151.
- Fargeas CA, Corbeil D, Huttner WB (2003) AC133 antigen, CD133, prominin-1, prominin-2, etc.: prominin family gene products in need of a rational nomenclature. *Stem Cells* **21**, 506–508.
- Fargeas CA, Fonseca AV, Huttner WB, Corbeil D (2006) Prominin-1 (CD133) – from progenitor cells to human diseases. *Future Lipidol.* **1**, 213–225.
- Gallacher L, Murdoch B, Wu DM, Karanu FN, Keeney M, Bhatia M (2000) Isolation and characterization of human CD34(–)Lin(–) and CD34(+)Lin(–) hematopoietic stem cells using cell surface markers AC133 and CD7. *Blood* **95**, 2813–2820.
- Gehling UM, Ergun S, Schumacher U, Wagener C, Pantel K, Otte M, Schuch G, Schafhausen P, Mende T, Kilic N, Kluge K, Schafer B, Hossfeld DK, Fiedler W (2000) *In vitro* differentiation of endothelial cells from AC133-positive progenitor cells. *Blood* **95**, 3106–3112.
- Gordon PR, Leimig T, Babarin-Dorner A, Houston J, Holladay M, Mueller I, Geiger T, Handgretinger R (2003) Large-scale isolation of CD133⁺ progenitor cells from G-CSF mobilized peripheral blood stem cells. *Bone Marrow Transplant.* **31**, 17–22.
- Goussetis E, Theodosaki M, Paterakis G, Peristeri J, Petropoulos D, Kitra V, Papassarandis C, Graphakos S (2000) A functional hierarchy among the CD34⁺ hematopoietic cells based on *in vitro* proliferative and differentiative potential of AC133⁺CD34(bright) and AC133(dim)/CD34⁺ human cord blood cells. *J. Hematother. Stem Cell Res.* **9**, 827–840.
- Kobari L, Giarratana MC, Pflumio F, Izac B, Coulombel L, Douay L (2001) CD133⁺ cell selection is an alternative to CD34⁺ cell selection for *ex vivo* expansion of hematopoietic stem cells. *J. Hematother. Stem Cell Res.* **10**, 273–281.
- Koehl U, Zimmermann S, Esser R, Sorensen J, Gruttner HP, Duchscherer M, Seifried E, Klingebiel T, Schwabe D (2002) Autologous transplantation of CD133 selected hematopoietic progenitor cells in a pediatric patient with relapsed leukemia. *Bone Marrow Transplant.* **29**, 927–930.
- Krause DS, Fackler MJ, Civin CI, May WS (1996) CD34: structure, biology, and clinical utility. *Blood* **87**, 1–13.
- Kuci S, Wessels JT, Bühning HJ, Schilbach K, Schumm M, Seitz G, Löffler J, Bader P, Schlegel PG, Niethammer D, Handgretinger R (2003) Identification of a novel class of human adherent CD34⁺ stem cells that give rise to SCID-repopulating cells. *Blood* **101**, 869–876.
- Lang P, Bader P, Schumm M, Feuchtinger T, Einsele H, Fuhrer M, Weinstock C, Handgretinger R, Kuci S, Martin D, Niethammer D, Greil J (2004) Transplantation of a combination of CD133⁺ and CD34⁺ selected progenitor cells from alternative donors. *Br. J. Haematol.* **124**, 72–79.
- Matsumoto K, Yasui K, Yamashita N, Horie Y, Yamada T, Tani Y, Shibata H, Nakano T (2000) *In vitro* proliferation potential of AC133 positive cells in peripheral blood. *Stem Cells* **18**, 196–203.
- Miraglia S, Godfrey W, Yin AH, Atkins K, Warnke R, Holden JT, Bray RA, Waller EK, Buck DW (1997) A novel five-transmembrane hematopoietic stem cell antigen: isolation, characterization, and molecular cloning. *Blood* **90**, 5013–5021.
- Pasino M, Lanza T, Marotta F, Scarso L, De Biasio P, Amato S, Corcione A, Pistoia V, Mori PG (2000) Flow cytometric and functional characterization of AC133⁺ cells from human umbilical cord blood. *Br. J. Haematol.* **108**, 793–800.
- Quirici N, Soligo D, Caneva L, Servida F, Bossolasco P, Deliliers GL (2001) Differentiation and expansion of endothelial cells from human bone marrow CD133(+) cells. *Br. J. Haematol.* **115**, 186–194.

- Torrente Y, Belicchi M, Sampaolesi M, Pisati F, Meregalli M, D'Antona G, Tonlorenzi R, Porretti L, Gavina M, Mamchaoui K, Pellegrino MA, Furling D, Mouly V, Butler-Browne GS, Bottinelli R, Cossu G, Bresolin N (2004) Human circulating AC133(+) stem cells restore dystrophin expression and ameliorate function in dystrophic skeletal muscle. *J. Clin. Invest.* **114**, 182–195.
- Weigmann A, Corbeil D, Hellwig A, Huttner WB (1997) Prominin, a novel microvilli-specific polytopic membrane protein of the apical surface of epithelial cells, is targeted to plasmalemmal protrusions of non-epithelial cells. *Proc. Natl. Acad. Sci. USA* **94**, 12425–12430.
- de Wynter EA, Buck D, Hart C, Heywood R, Coutinho LH, Clayton A, Rafferty JA, Burt D, Guenechea G, Bueren JA, Gagen D, Fairbairn LJ, Lord BI, Testa NG (1998) CD34⁺AC133⁺ cells isolated from cord blood are highly enriched in long-term culture-initiating cells, NOD/SCID-repopulating cells and dendritic cell progenitors. *Stem Cells* **16**, 387–396.
- Yin AH, Miraglia S, Zanjani ED, Almeida-Porada G, Ogawa M, Leary AG, Olweus J, Kearney J, Buck DW (1997) AC133, a novel marker for human hematopoietic stem and progenitor cells. *Blood* **90**, 5002–5012.