

## Brief report

## Human and rhesus macaque hematopoietic stem cells cannot be purified based only on SLAM family markers

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Various combinations of antibodies directed to cell surface markers have been used to isolate human and rhesus macaque hematopoietic stem cells (HSCs). These protocols result in poor enrichment or require multiple complex steps. Recently, a simple phenotype for HSCs based on cell surface markers from the signaling lymphocyte activation molecule (SLAM) family of receptors has been reported in the mouse. We examined the possibility of using the SLAM

markers to facilitate the isolation of highly enriched populations of HSCs in humans and rhesus macaques. We isolated SLAM (CD150<sup>+</sup>CD48<sup>-</sup>) and non-SLAM (not CD150<sup>+</sup>CD48<sup>-</sup>) cells from human umbilical cord blood CD34<sup>+</sup> cells as well as from human and rhesus macaque mobilized peripheral blood CD34<sup>+</sup> cells and compared their ability to form colonies in vitro and reconstitute immune-deficient (nonobese diabetic/severe combined immunodeficiency/

interleukin-2  $\gamma$ c receptor<sup>null</sup>, NSG) mice. We found that the CD34<sup>+</sup> SLAM population contributed equally or less to colony formation in vitro and to long-term reconstitution in NSG mice compared with the CD34<sup>+</sup> non-SLAM population. Thus, SLAM family markers do not permit the same degree of HSC enrichment in humans and rhesus macaques as in mice. (*Blood*. 2011;117(5):1550-1554)

## Introduction

The search for hematopoietic stem cell (HSC)-specific surface markers has been a central question for functional stem cell studies and for the development of clinical applications, including transplantation and gene therapy. In mice, using a complex combination of positive and negative selection for 10 to 12 surface markers (Lin<sup>-</sup>Sca-1<sup>+</sup>c-kit<sup>+</sup>Thy-1<sup>lo</sup>), 1 in 4.9 cells provides long-term reconstitution after intravenous injection.<sup>1</sup> However, the use of these complex sets of markers, alone or in combination with the established isolation scheme based on Hoechst dye efflux (side population),<sup>2</sup> is incompatible with in situ histologic analyses.

Recently, a simple and broadly applicable method to isolate mouse HSCs has been developed based on expression of cell surface markers, which are members of the signaling lymphocyte activation molecule (SLAM) family, including CD150, CD48, and CD244.<sup>1</sup> In transplantation assays, 1 in 4.8 CD150<sup>+</sup>CD48<sup>-</sup> bone marrow (BM) cells provides long-term, multilineage reconstitution in recipient mice, similar to the enrichment obtained in the complex Lin<sup>-</sup>Sca-1<sup>+</sup>c-kit<sup>+</sup>Thy-1<sup>lo</sup> population.<sup>1</sup> This same strategy has been successfully applied to enrich HSCs from mouse cyclophosphamide/granulocyte-colony stimulating factor (G-CSF)-mobilized cells,<sup>3</sup> mouse fetal liver,<sup>4</sup> as well as from the BM of various strains of mice<sup>5</sup> and older mice.<sup>3</sup> Recent data examining the overlap between SLAM family member expression with the Hoechst dye efflux

side population in conjunction with canonical HSC cell surface markers have confirmed the potential of CD150<sup>+</sup> selection for mouse HSC enrichment.<sup>6</sup> Although it has been suggested that some HSC activity may also be present in the CD150<sup>-</sup> cell fraction of mouse cells,<sup>6</sup> data from multiple groups indicate that there is little or no long-term HSC activity in the CD150<sup>-</sup> fraction of mouse hematopoietic cells.<sup>1,3,4,7-10</sup>

In humans, the CD34 cell surface marker is largely used in clinical applications for isolation of HSCs and progenitor cells, and as a predictor of graft HSC content in transplants. The combination CD34<sup>+</sup>CD38<sup>-</sup> provides further enrichment (1 in 600 to 1 in 3500),<sup>11</sup> but the heterogeneity of this population precludes studies requiring levels of purity achieved in mouse models, such as comparing gene expression profiles or in situ histologic analyses. Given the use of SLAM receptors for identifying long-term repopulating HSCs in mice,<sup>1,3,4,7-10</sup> we hypothesized that this strategy may similarly facilitate the isolation of highly enriched populations of HSCs in humans and nonhuman primates. In this study, we isolated SLAM (CD150<sup>+</sup>CD48<sup>-</sup>) and non-SLAM (not CD150<sup>+</sup>CD48<sup>-</sup>) cells from human umbilical cord blood (UCB) cells as well as human and primate cytokine mobilized peripheral blood (MPB) cells and compared their ability to form colonies in vitro and to reconstitute immune-deficient (nonobese diabetic/severe combined immunodeficiency/interleukin-2  $\gamma$ c receptor<sup>null</sup>, NSG) mice.

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## Methods

### Collection of hematopoietic cells from healthy donors and from rhesus macaques

Human MPB CD34<sup>+</sup> cells were obtained from 3 healthy volunteers (donor 1, 25-year-old woman; donor 2, 21-year-old man; and donor 3, 24-year-old man) after informed consent in accordance with the Declaration of Helsinki, under an institutional review board-approved clinical protocol. The donors received 5 days of G-CSF (filgrastim; Amgen) 10 µg/kg and underwent leukapheresis and CD34<sup>+</sup> cell enrichment as previously described.<sup>12</sup> The CD34<sup>+</sup> cells were cryopreserved before isolation of SLAM (CD150<sup>+</sup>CD48<sup>-</sup>) and non-SLAM (not CD150<sup>+</sup>CD48<sup>-</sup>) cells.

Human UCB samples were obtained from the National Heart, Lung and Blood Institute Biologic Specimen and Data Repository Coordinating Center. CD34<sup>+</sup> cells were collected from 8 pooled UCB samples. Each cryopreserved UCB unit was thawed at 37°C and washed in normal saline buffer containing 5% dextran (Hospira) and 2.5% human serum albumin (Baxter Healthcare). Cells were resuspended in normal saline containing 1% human serum albumin, pooled, and CD34<sup>+</sup> cells were enriched as previously described<sup>12</sup> before isolation of SLAM and non-SLAM cells.

The rhesus macaque (*Macaca mulatta*) used in these studies (RQ6566, 4-year-old female) was housed and handled in accordance to guidelines outlined in a protocol approved by the Animal Care and Use Committee of the National Heart, Lung and Blood Institute. Stem cell mobilization and apheresis were as described.<sup>13</sup> The CD34<sup>+</sup> cells had a purity of 90% and were used without prior cryopreservation for isolation of SLAM and non-SLAM cells.

### Isolation of SLAM and non-SLAM cells

Human and rhesus macaque SLAM and non-SLAM cells were isolated using human anti-CD150-fluorescein isothiocyanate (clone A12, eBioscience) and anti-CD48-RPE (clone MEM102, AbD Serotec) monoclonal antibodies. Cell sorting was performed using a FACSVantage cell sorter (BD Biosciences). Gates were defined strictly using isotype controls to eliminate any potential overlap between CD150<sup>+</sup> and CD150<sup>-</sup> cells.

### NSG mouse repopulation assay

Sublethally irradiated (300 cGy) 8-week-old NSG mice were transplanted by tail-vein injection with  $1 \times 10^3$ ,  $1 \times 10^4$ ,  $1 \times 10^5$ , or  $1 \times 10^6$  human SLAM cells, non-SLAM cells, or unfractionated CD34<sup>+</sup> cells. Mice transplanted with less than  $1 \times 10^5$  cells also received  $1 \times 10^5$  CD34<sup>-</sup> carrier cells (99.2% purity). At week 8 after transplantation, mice were killed and BM mononuclear cells were harvested from both femurs and tibias. Aliquots were stained with human CD45-phycoerythrin (PE), CD20-PE, CD3-PE, CD36-PE, and CD34-PE antibodies (all from BD Biosciences) and analyzed on a Coulter FC500 analyzer (Beckman Coulter) for detection of human cell engraftment.

### CFU assay

For colony-forming unit (CFU) assays,  $2 \times 10^5$  transplanted mouse BM cells were seeded in duplicates in 1 mL methylcellulose media (GF+H4435, Stem Cell Technologies) at 37°C in 5% CO<sub>2</sub>. Colonies of more than 50 cells were scored after 10 to 14 days of incubation.

## Results and discussion

Cell surface receptors of the SLAM family, including CD150, CD244, and CD48, were found to be differentially expressed among mouse HSCs (CD150<sup>+</sup>CD244<sup>-</sup>CD48<sup>-</sup>) and more restricted progenitors (CD150<sup>+</sup>CD244<sup>+</sup>CD48<sup>+</sup>).<sup>1</sup> Using CFU assays and transplantation studies in NSG mice, we investigated whether SLAM markers could also be used to distinguish human progeni-

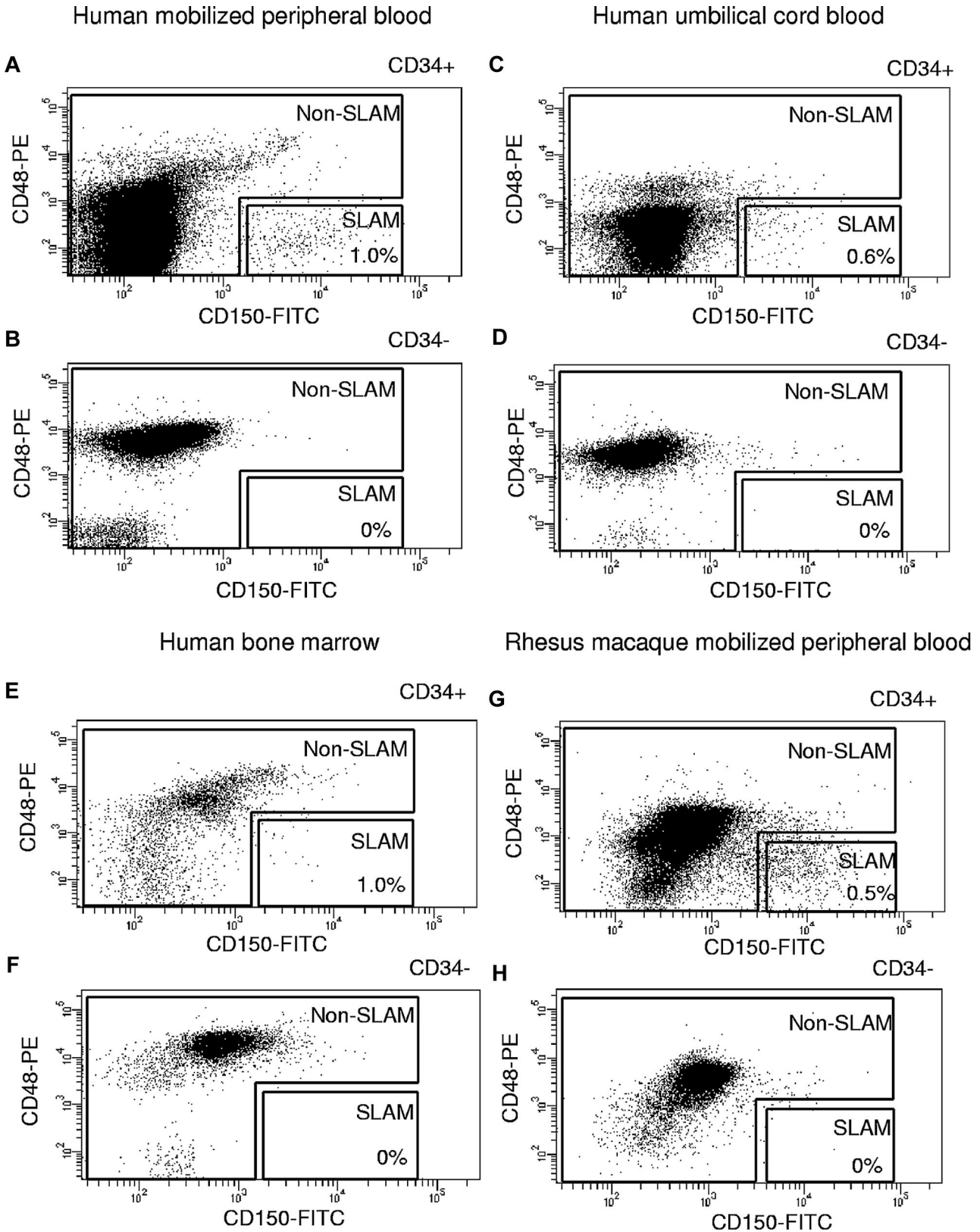
tors and more primitive repopulating cells (referred to as human SCID repopulating cells [hSRCs]<sup>11</sup>).

Because human CD150<sup>+</sup>CD48<sup>-</sup> cells were uniformly CD244<sup>-</sup>,<sup>1</sup> we omitted the CD244 marker and tested the *in vivo* repopulating and colony-forming potential of CD150<sup>+</sup>CD48<sup>-</sup> cells (SLAM cells) compared with non-SLAM cells, which included all viable cells outside the SLAM gate (not CD150<sup>+</sup>CD48<sup>-</sup>). These 2 populations were first sorted from human G-CSF-MPB cells derived from 3 normal volunteers and pre-enriched in CD34<sup>+</sup> cells (99% purity) to facilitate sorting of the rare CD150<sup>+</sup>CD48<sup>-</sup> cells. Using stringent gates defined with isotype controls, we found that 0.4% to 1.1% of human CD34<sup>+</sup> cells were CD150<sup>+</sup>CD48<sup>-</sup> (Figure 1A). Variable percentages of the SLAM population were in the CD38<sup>-</sup> fraction (donor 1, 8%; donor 2, 94%; and donor 3, 87%). No significant numbers of SLAM cells were detected in the CD34<sup>-</sup> fraction in any donor (Figure 1B). Therefore, the pre-enrichment step in CD34<sup>+</sup> cells performed before sorting cells based on the SLAM markers does not result in loss of SLAM cells. Similar results were obtained using human UCB cells (Figure 1C-D), human BM cells (Figure 1E-F), and rhesus macaque MPB cells (Figure 1G-H), indicating a consistent pattern of CD150 and CD48 expression in various sources of hematopoietic cells from large animals.

In CFU assays, SLAM, non-SLAM, and unfractionated CD34<sup>+</sup> cell populations obtained from human G-CSF MPB and human UCB gave rise to colonies of similar size, phenotype, and frequency (supplemental Figure 1A and supplemental Figure 1B, respectively, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article) after 10 to 14 days in culture. When rhesus macaque-mobilized CD34<sup>+</sup> cells were used in CFU assays, the size, phenotype, and number of colonies obtained from SLAM and non-SLAM populations were also similar to the CFUs derived from unfractionated CD34<sup>+</sup> cells (supplemental Figure 1C).

Surprisingly, both SLAM and non-SLAM populations derived from human MPB CD34<sup>+</sup> cells from 3 independent donors had *in vivo* repopulating potential after intravenous transplantation in NSG mice, and levels of human cell engraftment based on CD45 cell surface expression were similar to those obtained with unfractionated CD34<sup>+</sup> cells (Figure 2A-E). The human SCID repopulating cell activity in each fraction had a comparable ability to repopulate mice with multiple hematopoietic lineages (data not shown). CD34<sup>+</sup> SLAM cells derived from human UCB provided only minimal contribution to long-term hematopoietic reconstitution in NSG mice compared with non-SLAM CD34<sup>+</sup> cells and unfractionated CD34<sup>+</sup> cells (Figure 2F-J). Rhesus SCID repopulating cells were also detected in both SLAM and non-SLAM populations derived from MPB CD34<sup>+</sup> cells, and levels of engraftment were similar to those obtained after transplantation of unfractionated CD34<sup>+</sup> cells (supplemental Figure 2). These data confirm that both human and rhesus macaque primitive repopulating cells from various hematopoietic sources are partially contained within the SLAM fraction of cells, but SCID repopulating cells cannot be isolated based only on SLAM family markers.

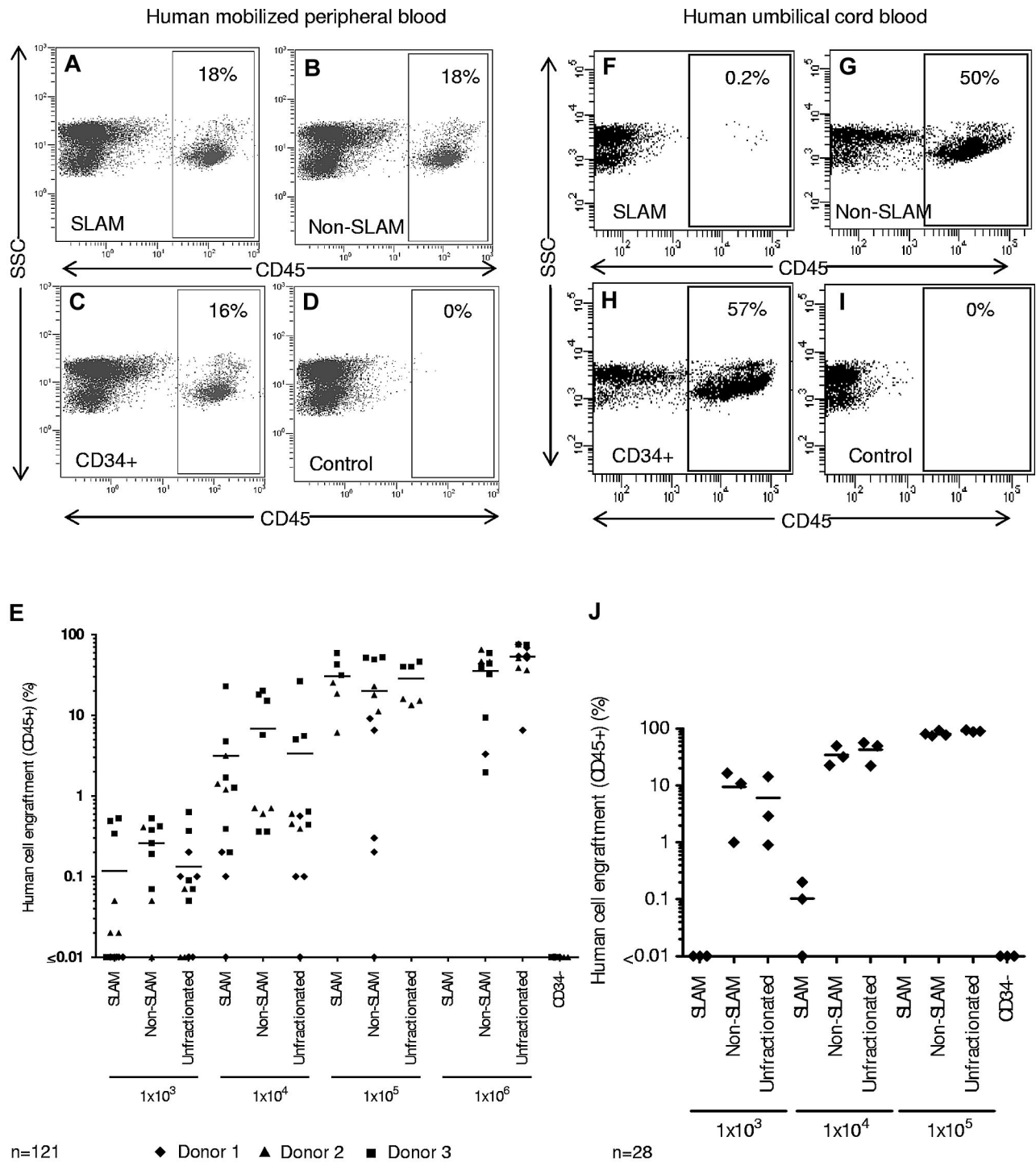
To rule out the possibility that these results could be explained by the existence of other post-translationally modified forms of CD150 present on human HSCs not recognized by the antibodies used in this study, we performed real-time reverse-transcriptase polymerase chain reaction to assess CD150 expression on human MPB (n = 3) and UCB (n = 3) CD34<sup>+</sup>CD38<sup>-</sup> cells. CD150 expression was 10-fold lower in this population compared with unfractionated mononuclear cells. This contrasts with our ability to detect enrichment of CD150 RNA in mouse HSCs. In addition,



**Figure 1.** Cell sorting experiments of SLAM (CD150<sup>+</sup>CD48<sup>-</sup>) and non-SLAM (not CD150<sup>+</sup>CD48<sup>-</sup>) populations. Human G-CSF MPB CD34<sup>+</sup> cells (A) and CD34<sup>-</sup> cells (B). Human UCB CD34<sup>+</sup> cells (C) and CD34<sup>-</sup> cells (D). Human BM CD34<sup>+</sup> cells (E) and CD34<sup>-</sup> cells (F). Rhesus macaque MPB CD34<sup>+</sup> cells (G) and CD34<sup>-</sup> cells (H). No significant numbers of SLAM cells are detected in the CD34<sup>-</sup> population from any hematopoietic source.

using flow cytometry, we were unable to detect increased staining on human CD34<sup>+</sup>CD38<sup>-</sup> cells using multiple CD150 antibodies (data not shown).

In contrast to mouse studies, our data in humans and rhesus macaques using UCB and MPB cells indicate that SCID repopulating cells and progenitor cells can be found in both SLAM and



**Figure 2.** Summary of human cell engraftment based on CD45 cell surface expression in the BM of nonobese diabetic/severe combined immunodeficiency/interleukin-2  $\gamma$  c receptor<sup>null</sup> mice transplanted with SLAM (CD150<sup>+</sup>CD48<sup>-</sup>), non-SLAM (not CD150<sup>+</sup>CD48<sup>-</sup>), or unfractionated CD34<sup>+</sup> cells. Representative flow cytometry analysis after transplantation of  $1 \times 10^5$  SLAM (A), non-SLAM (B), or unfractionated human CD34<sup>+</sup> cells (C) derived from G-CSF MPB. (D) Control mice were injected intravenously with an equal volume of saline. (E) Summary of human cell engraftment after transplantation of G-CSF MPB cells from 3 independent donors in NSG mice. Each symbol represents one mouse, and the horizontal lines indicate the mean levels of human cell engraftment (n = 121 mice). Representative flow cytometry experiment after transplantation of  $1 \times 10^4$  SLAM (F), non-SLAM (G), or unfractionated human CD34<sup>+</sup> cells (H) derived from 8 pooled cord blood samples. (I) Control animals were transplanted with an equal volume of saline. (J) Summary of human cell engraftment after transplantation of cord blood derived cells in NSG mice (n = 28 mice).

non-SLAM populations based on the available antibodies against human and rhesus macaque CD150 and CD48. Therefore, these combinations of antibodies against SLAM markers are not suitable for purification or in situ histologic analyses of HSCs in large mammals. The results derived from the functional studies presented here correlate with results of recently published flow

cytometry analyses showing that the patterns of expression of the SLAM family receptors differ between mice and humans.<sup>14</sup> Sintès et al<sup>14</sup> hypothesized, based on expression patterns, that the most primitive human repopulating cells may be predominantly CD150<sup>+</sup>CD48<sup>+</sup>, an observation contrasting with findings in mice<sup>1</sup> where HSCs are enriched in the CD150<sup>+</sup>CD48<sup>-</sup> population. This

observation is not uncommon and is evocative of the CD34 surface marker expression detected on the majority of human HSCs but absent on quiescent mouse HSCs.<sup>15-17</sup> It is also reminiscent of the absence of the differentiation marker CD38 on human HSCs<sup>11,18-21</sup> and its presence on mouse HSCs.<sup>22-26</sup> Additional transplantation studies will be useful to further characterize the repopulating potential of the CD150<sup>+</sup>CD48<sup>+</sup> population in large mammals.

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## Authorship

Contribution: A.L. and M.S. designed and performed the experimental procedures and analyzed the data; A.L. wrote the manuscript; M.S. contributed to the editing; M.W., S.A., and B.I. performed some of the transplantation studies; K.K. sorted the various cell populations; and C.E.D. and S.J.M. designed the experiments and edited the manuscript.

Conflict-of-interest disclosure: S.J.M. is an author on a patent for the use of SLAM family markers for the isolation of mammalian HSCs. The remaining authors declare no competing financial interests.

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