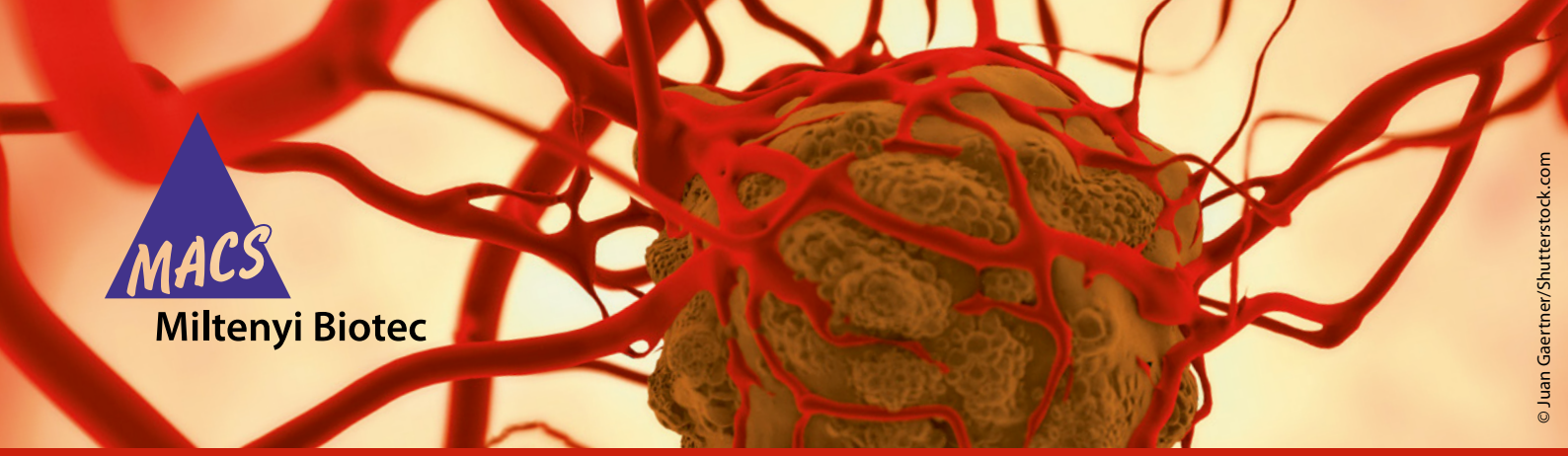

Separation and analysis

Mouse cell depletion

Purification of human hematopoietic cells from xenografts for the assessment of functional and genomic identity

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Background

Xenotransplantation models have provided advancements in the ability to model the initiation and progression of human malignancies and study the functional outputs of normal cells. This is especially true in the study of human hematopoiesis in which primary human cells are transplanted into immune-deficient mice. The potential of the xenograft model was first described for normal hematopoiesis in the 1980's and has since been adapted for the study of leukemia[1–3]. Transplantation of human primary acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) cells can generate robust human grafts which phenotypically resemble the patient disease and demonstrate robust engraftment, often correlating with poor patient prognosis[2–4]. Furthermore, the potential of xenograft assays to model human leukemic initiation has been highlighted through the recapitulation of human disease in immune-deficient mice by the transplantation of human lineage depleted cord blood cells transduced with oncogenes[5–8]. To further study the functional properties and genomic relationship of xenografted cells to the transplanted primary patient sample, depletion of mouse cells is necessary. The Mouse Cell Depletion Kit and the autoMACS® Pro Separator can be used to deplete mouse cells from the bone marrow and spleens of xenografts with high purity. Purified cells were then used directly in downstream *in vitro* cultures and DNA isolations without having to worry about any major mouse contribution in these assays. This note describes the isolation of human tumor cells from xenograts using the Mouse Cell Depletion Kit in combination with the autoMACS Pro Separator.

Materials and methods

Materials

- Mouse Cell Depletion Kit
- autoMACS Pro Separator
- FcR Blocking Reagent
- Labeling Check Reagent
- CD45 antibodies, human

For a detailed protocol, please refer to the respective data sheet.

Methods

Tissue collection

Femurs and tibias were collected from NSG xenografts transplanted intrafemurally with either human umbilical cord blood or human acute lymphoblastic leukemia cells. Bone marrow cells were isolated by either flushing the bones with media or crushing the bones with a mortar and pestle. Spleens were collected and cells were dissociated into a single-cell suspension by mechanical disruption. Tissues were analyzed for human engraftment by performing flow cytometry for human CD45 and then subsequently frozen.

Depletion of mouse cells from xenograft tissues/bone marrow

Bone marrow and spleen cells were thawed and counted. Human cells were purified from the xenograft bone marrows and spleens by negative selection using the Mouse Cell Depletion Kit on the autoMACS Pro Separator and the program "Depletes" according to the standard protocol with the addition of the FcR Blocking Reagent.

Analysis of target cells or analysis of cell separation

Untouched human cells were analyzed for purity by flow cytometry by staining for human CD45 and the Labeling Check Reagent. Viability was assessed using propidium iodide. Total cells prior to purification and mouse cells after depletion were also analyzed.

Results

Bone marrow cells and cells from the spleen of xenografts were analyzed by flow cytometry for human engraftment by determination of the percentage of human CD45 positive cells. The frequencies of human engraftment ranged from 3–90% and included xenografts transplanted with ALL as well as human umbilical cord blood cells.

Figure 1A and 1B show the flow cytometry analysis of the bone marrow of two representative xenografts. Human cells were purified from the xenografts using the Mouse Cell Depletion Kit on the autoMACS Pro Separator which resulted in high purity human CD45$^+$ cells as seen in figure 1C–D and figure 2. The recovery of cells following purification was on average 60.1% of the expected cell yield (figure 3). Similar results were established using manual separation with LS Columns (data not shown). Human cells were also recovered from the spleens of xenografts with high purity (figure 4).

Conclusion

The present data show that the Mouse Cell Depletion Kit allows for the isolation of human cells from the bone marrow and spleen of xenografts with high purity using both the autoMACS Pro Separator or MACS® Columns without wash steps required. Recovered cells are pure and viable.

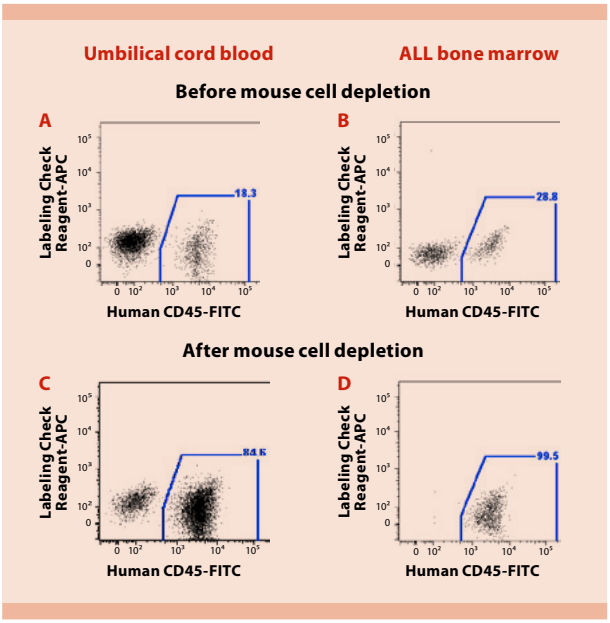

Figure 1: Representative flow cytometry analysis of xenografts transplanted with human umbilical cord blood (left panel) and human acute lymphoblastic leukemia bone marrow cells (right panel). Human cells were purified from the bone marrow of xenografts by negative selection using the Mouse Cell Depletion Kit. Purity of selection was analyzed for the umbilical cord blood before (A) and after purification (C) and acute lymphoblastic leukemia before (B) and after purification (D) by staining with the Labeling Check Reagent-APC and human CD45-FITC. Cells negative for the Labeling Check Reagent and human CD45 in umbilical cord blood mice most likely represent human erythrocytes.

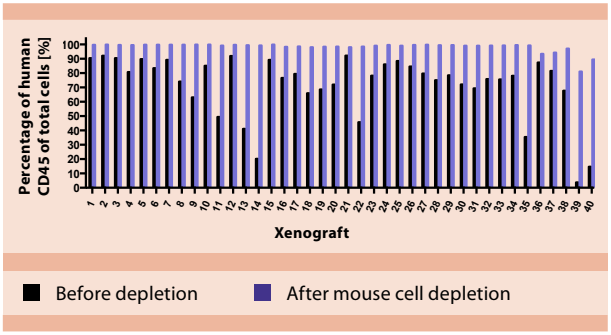

Figure 2: The Mouse Cell Depletion Kit results in high purity of human cells from xenografts with a wide range of initial human engraftment levels. Bone marrow cells of xenografts transplanted with human acute lymphoblastic leukemia (n=36) and human umbilical cord blood (n=4) were collected and purified by negative selection using the Mouse Cell Depletion Kit. Purity was assessed by an enrichment in the percentage of human CD45 cells.

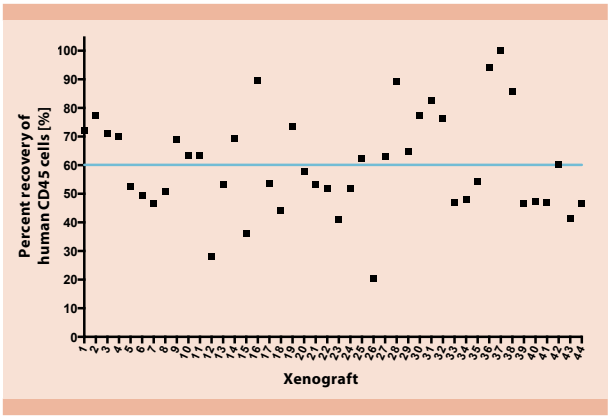

Figure 3: Cell recovery of human cells after purification with the Mouse Cell Depletion Kit. The percent recovery was calculated by determining the expected number of human cells based on initial human engraftment and cell numbers and comparing this to the total number and purity of cells after purification of xenografts transplanted with acute lymphoblastic leukemia (n=40) and umbilical cord blood (n=4). The line represents the average percent recovery of 60.1%.

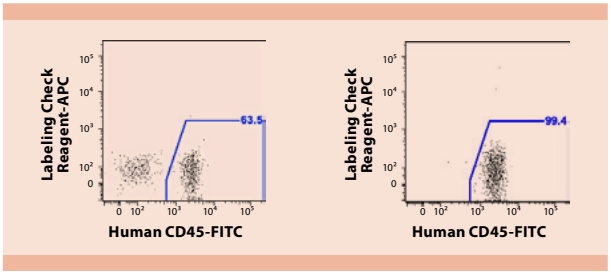

Figure 4: Representative flow cytometry analysis of the spleen of a xenograft transplanted with acute lymphoblastic leukemia. The Mouse Cell Depletion Kit also successfully purifies human cells from the spleens of xenografts as shown by the percentage of cells positive for human CD45 before (A) and after purification (B) of the spleen.

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Visit **www.miltenyibiotec.com/mcd** for more information on the Mouse Cell Depletion Kit.

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