Sensitive analysis and isolation of ROR1+ B cells for research on chronic lymphocytic leukemia

Introduction

The challenge of analyzing lymphoid cells in chronic lymphocytic leukemia research

Flow cytometric analysis of lymphoid cells from chronic lymphocytic leukemia (CLL) samples requires a complex panel of multiple markers. Most of these markers are expressed on both normal and malignant cells, and only characteristic marker expression profiles help distinguish between normal and malignant cells. Identification of new markers and the optimization of antibody panels for flow cytometric immunophenotyping would greatly enhance leukemia research.

Analysis of malignant B cells becomes especially difficult with samples containing only very low amounts of these cells, e.g., in minimal residual disease (MRD). Enrichment of malignant B cells from these samples would help overcome this obstacle. However, current techniques for the isolation of B cells do not allow for a distinction between malignant and normal cells. This drawback hampers the analysis of differential gene expression, for example.

ROR1 as a B cell marker in CLL research

ROR1 is a member of the receptor tyrosine kinase–like orphan receptor (ROR) family and has characteristics of an oncofetal antigen. ROR1 signaling increases cell survival via the wnt pathway[1]. The protein is expressed on B cells from CLL blood samples, but not from blood of healthy donors.[1,2,3] ROR1 is also expressed on various solid tumors[4], but not in major adult tissues apart from low levels in adipose tissue, on embryonic stem cells, and transiently at an early stage of B cell development[5]. Therefore, ROR1 is a potential marker to identify and isolate CLL B cells from blood samples for downstream analysis.

Tools for the analysis and isolation of ROR1+ B cells from CLL samples

Analysis of ROR1+ B cells in CLL samples

Miltenyi Biotec offers an Anti-ROR1 antibody, which increases sensitivity and specificity of flow cytometric B cell analysis in CLL research. It is available as a conjugate with PE, APC, or biotin. For functional studies, the antibody is provided in an unconjugated form, without toxic preservatives or endotoxins. Figure 1 shows a typical immunophenotyping experiment with a CLL sample. PBMCs were labeled with a panel of eight MACS® Antibodies and analyzed by flow cytometry using the following setup: violet laser (405 nm): CD20-VioBlue®; blue laser (488 nm): CD43-FITC, CD5-PE, CD79b-PerCP-Vio700™, CD19-PE-Vio770™; red laser (633 nm): Anti-ROR1-APC, CD3-APC-Vio770.

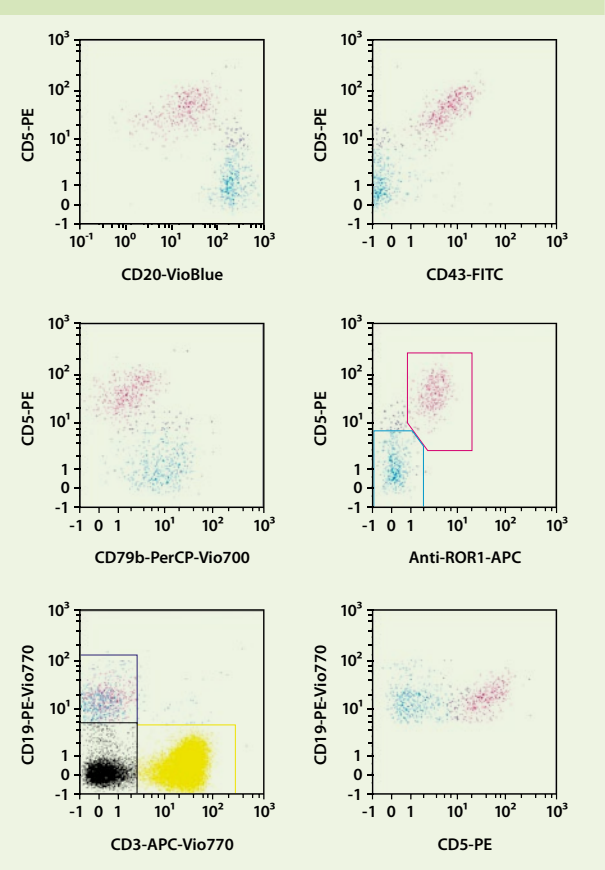

Figure 1: Identification of ROR1+ cells and other immune cells in PBMCs from a CLL sample. Cells were labeled with CD20-VioBlue, CD43-FITC, CD5-PE, CD79b-PerCP-Vio700, CD19-PE-Vio770, Anti-ROR1-APC, and CD3-APC-Vio770, and analyzed on the MACSQuant Analyzer 10. CD19+ROR1+ B cells are shown in magenta, CD19+ROR1- B cells in light blue, T cells in yellow, and NK cells in black.

Isolation of ROR1⁺ cells from CLL samples

For the isolation of ROR1⁺ B cells, Miltenyi Biotec has developed the Anti-ROR1 MicroBead Kit. Using this kit, ROR1⁺ cells are indirectly magnetically labeled with an Anti-ROR1-PE antibody and Anti-PE MicroBeads. Subsequently, the labeled ROR1⁺ cells are isolated manually using LS or MS Columns, or automatically with the autoMACS® Pro Separator. The isolated ROR1⁺ B cells are then ready for any downstream analysis. ROR1⁺ cells can be enriched to high purities and numbers suitable for further research processing – even from samples with very low ROR1⁺ cell frequencies, e.g., in MRD (fig. 2).

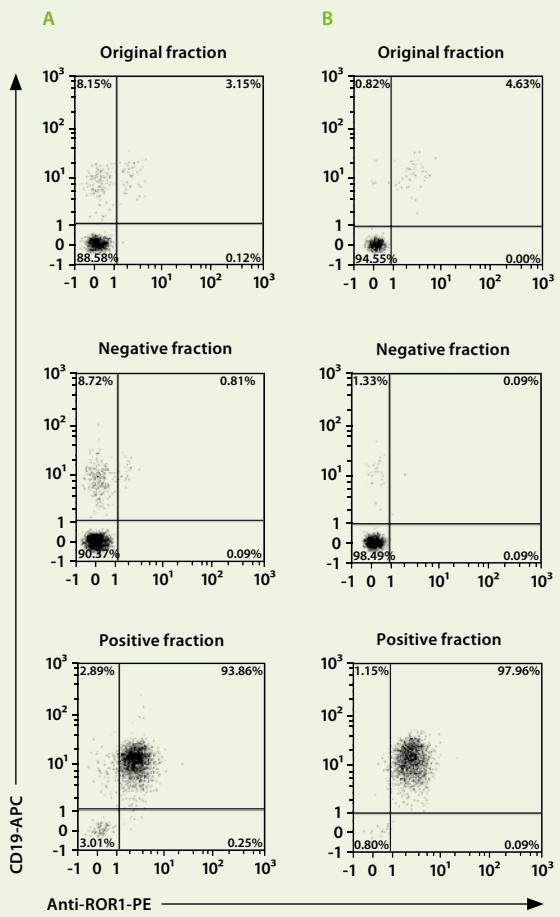

Figure 2: Enrichment of ROR1⁺ cells from PBMCs of a donor with MRD. ROR1⁺ cells were isolated using the Anti-ROR1 MicroBead Kit, either manually (A) or automatically using the autoMACS Pro Separator (B). Cells were fluorescently labeled with Anti-ROR1-PE and CD19-APC and analyzed by flow cytometry. Purities of the manually and automatically isolated cells amounted to 94% and 98%, and yields amounted to 45% and 97%, respectively.

Conclusion

- Anti-ROR1 antibodies enhance the sensitivity and specificity of flow cytometric immunophenotyping of CLL samples.
- Anti-ROR1 antibodies allow the identification and enumeration of B cells for CLL research.
- The Anti-ROR1 MicroBead Kit enables the enrichment of ROR1⁺ CLL B cells – even from samples containing only very low amounts of these cells, for example, in MRD.
- Isolation of ROR1⁺ cell populations to high purities facilitates research on the specific features and functions of CLL B cells.

References

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