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A Novel Preclinical Mouse Model of Chronic Lymphocytic Leukemia Driven by BCOR Loss in B Cells

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OBJECTIVES

- **To develop and characterize a novel mouse model with B-cell-specific BCOR deletion and evaluate its ability to spontaneously develop a CLL-like disease with aging.**

CONCLUSIONS

These findings establish CD19Bcor$^{-/-}$ mice as a novel preclinical model that recapitulates key features of human low-grade CLL. Our data demonstrate that BCOR loss in B cells is sufficient to drive a chronic lymphoproliferative disorder with CLL-like features, providing a unique platform to dissect the molecular consequences of BCOR inactivation and to explore therapeutic vulnerabilities in CLL.

INTRODUCTION

- Chronic lymphocytic leukemia (CLL) is a blood cancer marked by the accumulation of CD19+CD5+ cells resistant to apoptosis. Although treatments have improved, CLL remains incurable, highlighting the need for new therapeutic targets. Mutations in the tumor suppressor gene *BCOR* occur in about 2% of CLL cases and are often found alongside unmutated IGHV, trisomy 12, and *NOTCH1* mutations (1). BCOR loss enhances NOTCH1 activity and has been shown in the TCL1 mouse model to promote Richter Transformation (RT) (2).
- However, it is still unclear whether BCOR loss alone is sufficient to initiate CLL, pointing to an important gap in our understanding.

METHODS

- We generated a conditional knockout mouse model by crossing Bcor$^{flox/flox}$ female and Bcor$^{flox/y}$ male mice with CD19-Cre+ transgenic mice to achieve Bcor deletion specifically in CD19+ B cells (Hereafter, homozygous Bcor$^{flox/flox}$;Cre+ and hemizygous Bcor$^{flox/Y}$;Cre+ will be referred as CD19Bcor$^{-/-}$)
- Mice were longitudinally monitored, and peripheral blood (PB), bone marrow (BM), and spleen were systematically analyzed through complete blood counts, multiparametric flow cytometry, histopathological examination, and immunophenotyping.
- To assess clonality, CD19+CD5+ splenic B cells were sorted and analyzed for immunoglobulin heavy chain (IGHV) gene rearrangements.
- To evaluate disease transplantability, 1x10^7 splenic cells from aged CD19Bcor$^{-/-}$ mice were injected intravenously into NSG recipient mice.

RESULTS

At 18 months, CD19Bcor$^{-/-}$ mice showed B-cell lymphopenia, with fewer follicular and more marginal zone B cells, but no clear signs of cancer (2). From 22 to 28 months, they had significantly reduced survival compared to wild-type mice (Figure 1). White blood cell counts were higher in CD19Bcor$^{-/-}$ mice during late-stage follow-up. Flow cytometry analysis revealed a major expansion of CD19+CD5+ cells in blood, spleen, and bone marrow (Figure 2). Histological analysis showed that these cells had infiltrated multiple organs including the lung, liver, spleen, and kidney (Figure 3). IGHV rearrangement analysis confirmed monoclonality of the CD19+CD5+ cells (Figure 4). These findings were consistent with a low-grade CLL-like lymphoproliferative disorder. CLL-like cells from spleens were transplantable into NSG mice, which developed similar disease. In adoptive transfers, disease progression accelerated with each transplantation round (Figure 5A). Transferred cells retained the ability to infiltrate organs, mirroring the primary tumor behavior (Figure 5B).

Figure1: CD19Bcor$^{-/-}$ mice exhibit a reduced survival

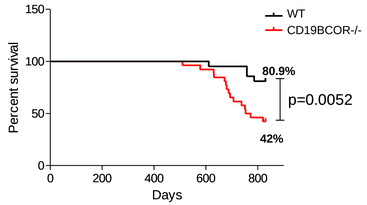

Figure1: Kaplan–Meier survival curves of 47 WT (female Bcor$^{flox/flox}$;Cre- and male Bcor$^{flox/Y}$;Cre−) and 47 CD19Bcor$^{-/-}$ (homozygous female Bcor$^{flox/Y}$;Cre− and hemizygous male Bcor$^{flox/Y}$;Cre+) mice.

Figure2: CD19Bcor$^{-/-}$ mice show a progressive accumulation of leukemic-like B cells CD19+CD5+

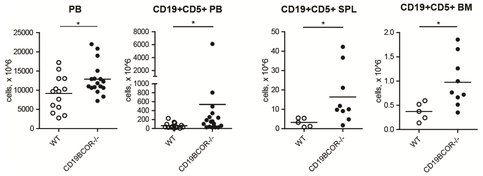

Figure 2: Left panel, WBC number of CD19Bcor$^{-/-}$ (n=17) mice compared to WT controls (n=14). p< 0.0371. Number of CD19+CD5+ cells in PB (middle left; n=17 CD19Bcor$^{-/-}$, 14 WT, p<0.0337), spleen (middle right; n=9 CD19Bcor$^{-/-}$, 5 WT, p<0.0190) and bone marrow (right; n=9 CD19Bcor$^{-/-}$, 5 WT, p<0.0013) of CD19Bcor$^{-/-}$ mice compared to WT controls from 22 to 28 months.

Figure 3: CD19Bcor$^{-/-}$ moribund mice showed a multiple organ infiltration

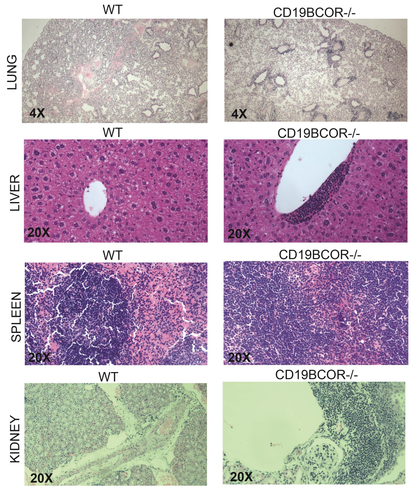

Figure 3: Representative images of Hematoxylin and Eosin (H&E) staining of lung (4× magnification), liver, spleen, and kidney (20× magnification) sections from WT (left panels) and CD19Bcor$^{-/-}$ (right panels) mice. Images were acquired using a UPlanApo 40×/0.85 NA objective on an Olympus BX-51 microscope.

Figure 4: Splenic CD19+CD5+ cells of CD19Bcor$^{-/-}$ are monoclonal

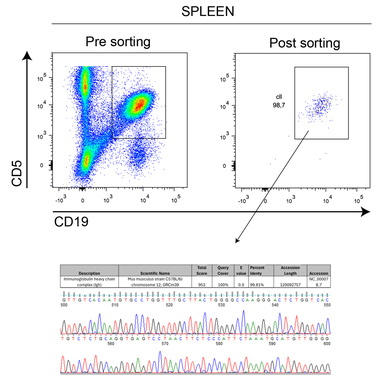

Figure 4: Upper panel, representative flow cytometry plots of splenic CD19+CD5+ cells pre and post sorting. Alignment results of the nucleotide sequence of J558VH gene (bottom panel) amplification in splenic sorted CD19+CD5+ cells from one representative BcorCD19$^{-/-}$ mouse, with the respective Sanger electropherogram, showing an unmutated clonal IGHV1 gene rearrangement (≥98% homology to germline). IgBlast Tool was used for the alignment of sequence against NCBI database

Figure 5: Adoptive transfer of CD19+CD5+ cells exhacerbates disease progression in transplanted mice.

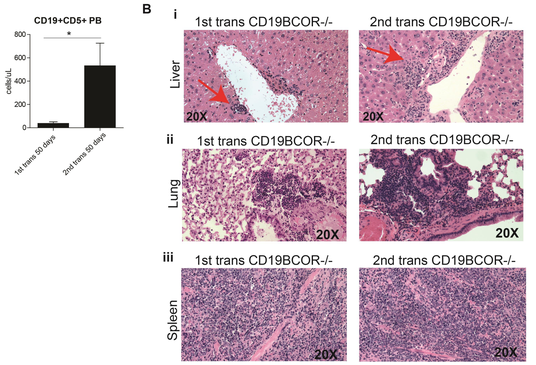

Figure 5: A) Number of CD19+CD5+ cells in PB, 50 days after the 1st round of transplantation compared to 50 days after the 2nd round of transplantation of CD19+CD5+ CD19Bcor$^{-/-}$ splenic cells. B) Representative images of H&E stained sections of liver (i), lung (ii) and spleen (iii) from BcorCD19$^{-/-}$ transplanted mice (1st round, left versus 2nd round, right) acquired at 20x magnification, using a UPlanApo 40×/0.85 NA objective, Olympus BX-51 microscope.

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DISCLOSURES

The authors declare no competing interests.