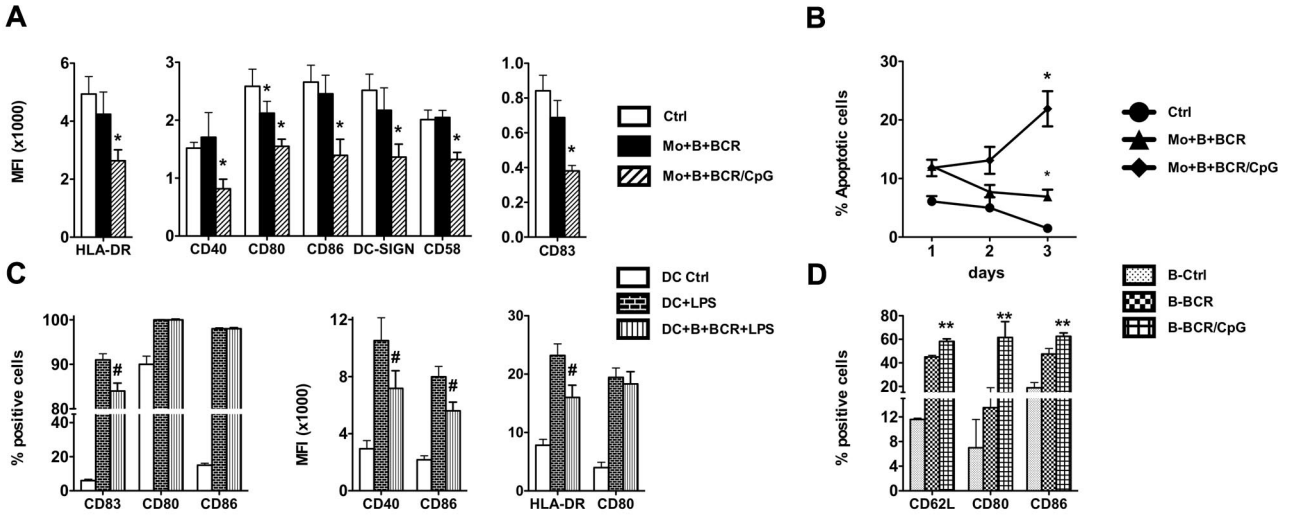

Figure 1. Regulation of human dendritic cells by B cells depends on the signals they receive. (A-B) Peripheral blood CD14$^+$ monocytes from healthy donors were cultured in the presence of GM-CSF (1000 IU/10^6 cells) and IL-4 (500 IU/10^6 cells) alone (Ctrl) or cytokines and BCR-activated (10 μg/mL of F(ab')$_2$ anti–human IgM) CD19$^+$ B cells (at ratio of 1:4; Mo + B + BCR) or cytokines and BCR + CpG-ODN-2006 (0.25μM; Mo + B + BCR/CpG) for 6 days. (A) Expression of DC surface markers (mean fluorescence intensities [MFI]) as analyzed by flow cytometry on CD20-negative cells (n = 5 experiments). (B) Percentage of annexin V$^+$ apoptotic DCs from days 1 to 3 after coculture with B cells (n = 3 experiments). (C) Five-day-old monocyte-derived immature DCs were cultured in the presence of GM-CSF and IL-4 alone (DC Ctrl) or stimulated with LPS (100 ng/mL; DC + LPS) or cocultured at 1:4 ratio with BCR-activated CD19$^+$ B cells in the presence of LPS (DC + B + BCR + LPS) for 48 hours to analyze the expression of surface markers (% positive cells and MFI) on CD20-negative cells. (n = 5 experiments). (D) CD19$^+$ B cells were either nonstimulated (B-Ctrl) or stimulated with BCR (B-BCR) or BCR + CpG (B-BCR/CpG) for 3 days and expression of surface markers (% positive cells) was analyzed (n = 3 experiments). The statistical significance as determined by 2-tailed paired Student *t* test is indicated (*$P < .05$ v/s Ctrl; #$P < .05$ v/s DC + LPS, and **$P < .05$ vs B-BCR).

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To the editor:

Rearrangement of *NOTCH1* or *BCL3* can independently trigger progression of CLL

Recent data indicate that *NOTCH1* mutations significantly increase the risk of CLL progression toward Richter syndrome (RS) and chemoresistance,[1,2] and that activation of *NOTCH1* at time of CLL diagnosis is an independent prognostic factor of poor survival.[1,3] We report here a case of CLL with a novel rearrangement of *NOTCH1* identified at the time of RS. The patient, a 58-year-old male, was diagnosed with CLL (unmutated *VH*) in RS in June 2003. Cytogenetic analysis and FISH on peripheral blood (PBL), bone marrow (BM), and lymph node (LN) cells showed 2 related clones: one with an isolated +12 and a second with +12 and dic(9;14)(q34;q32) (supplemental Table 1, available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article). FISH analysis of dic(9;14)(q34;q32) indicated that this aberration resulted in juxtaposition of 3′*IGH* and 5′*NOTCH1,* as evidenced by the loss of sequences telomeric to the breakpoints (Figure 1A-D). These imbalances were confirmed by array CGH (data not shown). The targeting of *NOTCH1* by dic(9;14) was evidenced by qRT-PCR analysis, which showed a 10-fold up-regulation of *NOTCH1* mRNA (Figure 1E) and a low expression of the neighboring genes (*GPSM11, CARD9, DNL2*). Immunoblotting of a cell lysate from LN with a NOTCH1 antibody recognizing active, cleaved NOTCH1 (Val1744) identified a band corresponding to activated intracellular NOTCH1 (Figure 1F), suggesting an additional truncating mutation. Indeed, sequence analysis identified a 2 basepair deletion, ΔCT7544–7545/P2515fs, in the nucleotide sequence encoding the PEST domain (Figure 1G). This mutation resulting in expression of a truncated intracellular *NOTCH1* allele is recurrent in T-ALL[4] and CLL.[1-3,5]

The patient was treated and achieved complete remission (supplemental Table 1). In 2007, however, CLL relapsed and an examination of BM identified a clone with a sole +12 negative for ΔCT7544–7545/P2515fs. Two years later, CLL progressed and BM revealed an evolved clone with complex aberrations including +12 and t(14;19)(q32;q13)/*IGH-BCL3* but lacking dic(9;14)(q34;q32). Of note, BM was again positive for ΔCT7544–7545/P2515fs. Despite treatment, 2 clones harboring +12, one with t(14;19) and a second with new additional karyotypic changes, were seen in the analyzed BM (06/2011) positive for ΔCT7544–7545/P2515fs. *TP53* disruption frequently associated with RS[6] was not observed in the analyzed

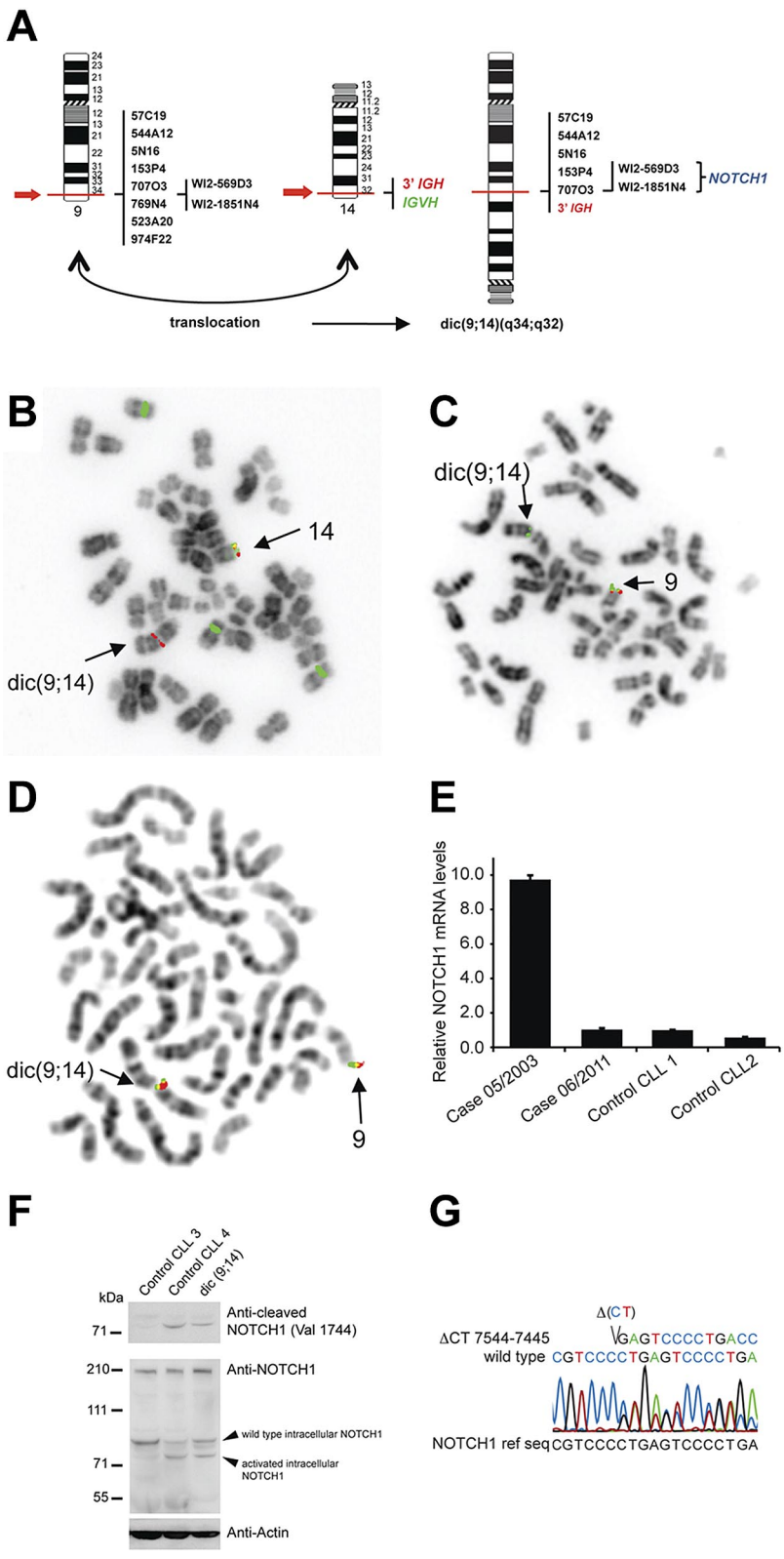

Figure 1. Genetic and molecular analysis of *NOTCH1* aberrations. (A) Graphic representation of dic(9;14)(q34;q32) with indicated probes applied for FISH mapping of both breakpoints and their hybridization pattern. (B-D) Examples of FISH analysis performed on a diagnostic LN sample with (B) LSI IGH dual color break apart probe and CEP12 (green), (C) RP11-707O3 (green) and RT11-769N4 (red), (D) WI2-569D3 (G248P80019F4, green) and WI2-1851N4 (G248P8957B2, red). BAC and fosmid clones were selected using the UCSC Genome Browser on Human May 2004 (NCBI35/hg17) Assembly. Note in panel B the presence of the 3′*IGH*/red signal on dic(9;14) and loss of the distal *IGVH*/green signal in cell with trisomy 12, in panel C hybridization of RP11-707O3 (green) with dic(9;14) and loss of the distal RT11-769N4 (red) sequences, and in panel D hybridization of both fosmids covering *NOTCH1* with dic.(9;14). (E) qRT-PCR analysis of *NOTCH1* mRNA expression levels. The patient was analyzed at 2 different timepoints, at diagnosis (05/2003; PBL; 57% of cells with dic(9;14)) and during disease evolution (06/2011; PBL; dic(9;14)-negative), and relative *NOTCH1* expression levels were compared with 2 control CLL samples (CLL1 and CLL2) with unmutated *VH* and trisomy12. (F) Western blot analysis of protein extract of diagnostic LN cells of the index case (15% of cells with dic(9;14)) and 2 control CLLs (unmutated *VH*, trisomy12); CLL3 with unmutated *NOTCH1* and CLL4 positive for ΔCT7544-7545/P2515fs. The top panel shows detection of active, cleaved NOTCH1 (Val 1744 antibody; Cell Signaling Technology) in CLL4 and the index case, but not in CLL3 cells. The bottom panel shows expression of NOTCH1 with a general anti-NOTCH1 antibody (c-20 Santa Cruz Biotechnology). Because of a low content of cells with dic(9;14) in the only available LN sample, overexpression of an activated form of NOTCH1 could not be evidenced. (G) Sanger chromatogram illustrating a heterozygous ΔCT7544-7545/P2515fs of the *NOTCH1* cDNA in the diagnostic BM sample. FISH images acquired with a 63×/1.4 oil immersion objective in an Axioplan 2 fluorescence microscope equiped with an Axiophot 2 camera (Carl Zeiss) and an Isis imaging system (MetaSystems). Images were imported directly into PowerPoint (Microsoft).

samples (supplemental Table 1). Four months later, the patient developed peripheral T-cell lymphoma, a rare recurrent event in CLL.[7] Altogether, the present case allows us to deduce the sequence of multiple genetic defects driving development and progression of CLL. An initial clone with +12, likely present at a presymptomatic CLL phase, later acquired an activating mutation of *NOTCH1*. Subsequent acquisition of dic(9;14)/*IGH-NOTCH1* triggered Richter transformation. After a few years, a persistent, chemorefractory *NOTCH1*-mutated clone underwent another hit, t(14;19)/*IGH-BCL3*, which initiated the second progression that was followed by a fatal CLL-unrelated T-cell lymphoma. Our findings confirm the risk of activating mutations of *NOTCH1* in Richter transformation of CLL,[1,2] particularly CLL with +12,[3] and highlight that a residual chemotherapy-

resistant *NOTCH1*-mutated clone is at risk of acquiring further progression-associated hits. Besides the known t(14;19)(q32;q13)/*IGH-BCL3*,[8-10] we also identified dic(9;14)(q34;q32)/*IGH-NOTCH1*, which so far has not been reported in B-cell leukemia/lymphoma, as a novel genomic aberration capable of triggering RS.

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Contribution: K.D.K. and I.W. designed and performed the research, analyzed data, and wrote and approved the manuscript; L.M. analyzed data and wrote and approved the manuscript; A.B. and C.G. treated the patient, contributed vital patient information, and approved the manuscript; J.F.F. performed array CGH, analyzed data, and approved the manuscript; P.V. analyzed data and wrote and approved the manuscript; and J.C. designed the research, analyzed data, and wrote and approved the manuscript.

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To the editor:

Persistently high quality of life conferred by coexisting congenital deficiency of terminal complement C9 in a paroxysmal nocturnal hemoglobinuria patient

Paroxysmal nocturnal hemoglobinuria (PNH) clone bears a *PIGA* mutation and fails to express glycosylphosphatidylinositol-linked membrane proteins such as complement-regulatory CD55 and CD59, leading to complement-mediated intravascular hemolysis and thrombosis. The advent of eculizumab, an inhibitor of terminal complement C5, provides good quality of life (QOL) by preventing hemolysis and thrombosis,[1,2] and may improve prognosis of PNH patients.[3] However, the safety of its long-term use for more than 10 years[1,2] and the pathogenesis of eculizumab-associated extravascular hemolysis have not been established.[4] For the hemolysis, steroid, splenectomy,[5] and C3-targeted therapy[6] have been proposed, despite their individual risks.[7] In 1980, we found a PNH patient with a coexisting congenital deficiency of C9, still the only case globally.[8] Presently, the patient is 78 years old and has kept a high QOL (no experience of massive hemolysis, thrombotic events, critical infection, or malignant diseases) for more than 31 years after the PNH diagnosis. Of note, the patient manifests very low levels of PNH hemolysis (Figure 1A-B) and marrow failure (Figure 1A). The high QOL may reflect that the C9-deficiency prevents membrane attack complex (C5b-9) formation but allows immune reactions by generation of C5a and C5b-8.[7] Currently, virtually all erythrocytes (and granulocytes) of the patient showed the PNH phenotype (Figure 1A-C). Whole blood cells had the same *PIGA* mutation in 1998 and 2011 (Figure 1D), indicating that the cells are of a single PNH clone. Marrow cells showed a normal karyotype. These findings support the concept that clonal hematopoiesis in PNH is a benign process. The clinical features suggest the safety and efficacy of long-term inhibition of terminal complement including C9 for even elderly PNH patients. Thus, we propose that C9 targeting is another option for PNH therapy.[2]

Hemosiderinuria (Figure 1E) is an indicator of intravascular hemolysis, probably induced by C5b-8 in the C9-deficient patient.[7]