and a subsequent risk of developing HL NS (Table 1).

Our findings are important for several reasons. We show for the first time that the risk for HL following a personal history of autoimmune disease may vary by HL subtype. Autoimmunity was associated with a significantly elevated risk for MC HL, but there were no significant associations with NS HL and the risk estimates were generally lower. Interestingly, we found a long latency from the date of autoimmune diagnosis to the date of MC HL (mean time =15.4 years) suggesting that long-term chronic immune stimulation may play a role in the causation of MC HL. Taken together, our observations support the hypothesis that the etiology of HL might differ by HL subtype. Also, we confirmed that Sjögren's syndrome is associated with a highly increased risk of HL.

We used high-quality data,[11] however, the nature of this study is hypothesis-generating and one has to interpret our findings with caution due to the low numbers of cases by subtype, hampering further stratified analyses.

In conclusion, our findings support a role for chronic immune stimulation in the etiology of HL. Our novel finding of a different risk pattern by HL subtypes suggests the operation of separate pathogenetic mechanisms and needs to be supported by other study groups.

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A novel dasatinib-sensitive *RCSD1-ABL1* fusion transcript in chemotherapy-refractory adult pre-B lymphoblastic leukemia with t(1;9)(q24;q34)

Dasatinib, a wide-spectrum tyrosine kinase inhibitor (TKI), has shown notable clinical activity in Philadelphia chromosome positive (Ph^+) acute lymphoblastic leukemia (ALL) patients carrying a 9;22 translocation and the resulting *BCR-ABL1* fusion gene.[1] In other subtypes of ALL, its clinical efficacy and putative molecular targets are unknown. In this report we describe a pre-B ALL patient, who had chemotherapy resistant, but dasatinib sensitive disease and a t(1;9)(q24;q34) translocation. Sequencing revealed a novel *RCSD1-ABL1* fusion transcript suggesting involvement of this fusion tyrosine kinase in leukemogenesis and sensitivity to dasatinib.

A 40-year old male was diagnosed with a pre-B ALL based on immunophenotyping ($CD19^+$, $CD22^+$, $CD10^+$, $cCD79a^+$, $nTdT^+$, $CD34^+$, $CD38^+$, $HLA\text{-}DR^+$). The bone marrow (BM) aspirate showed 80% blast cells. The cytogenetic analysis revealed a t(1;9)(q24;q34), described in more detail below. The patient received a modified CVAD induction therapy. After 2 induction courses, the BM had residual disease with 50% of leukemic blasts. At day 10 from the start of the 3rd induction regimen, dasatinib was introduced at a dose of 140 mg once daily. Two weeks later morphological remission was confirmed, and only minimal residual disease (1.5% of leukemic cells) was detectable by FISH. The first consolidation therapy consisted of dasatinib 140 mg once daily and high-dose methotrexate. BM at four weeks post consolidation showed complete morphological and cytogenetic remission (CCyR) (1,020 cells analyzed by FISH). Dasatinib was continued until an allogeneic hematopoietic stem cell transplantation (HSCT) was performed four months after diagnosis. After transplantation, no TKI therapy was used.

At 12 months from HSCT, the patient experienced a cytogenetic relapse and a month later a hematologic relapse. Immunosuppressive drugs were discontinued, and the patient received a re-induction therapy with

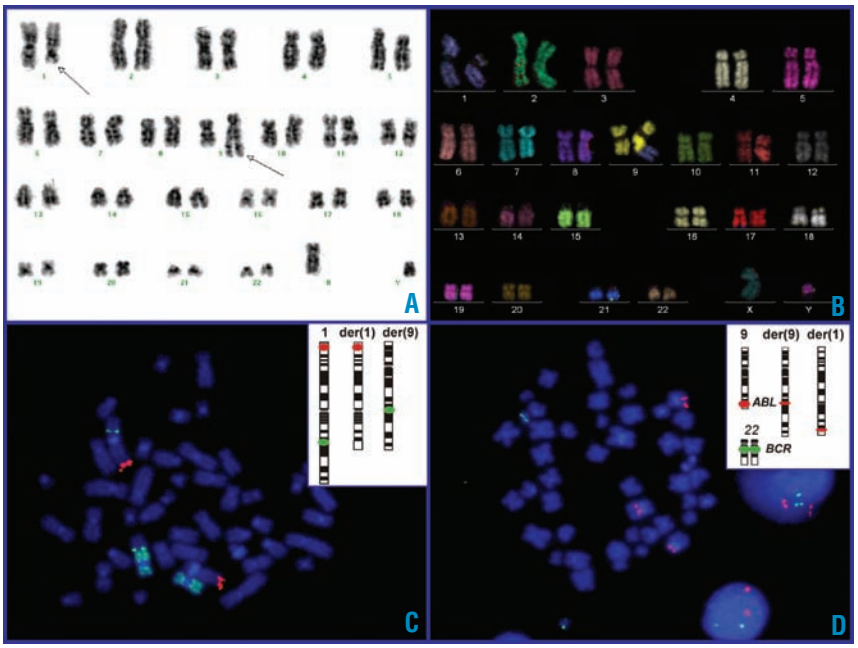

Figure 1. Cytogenetic characterization of the t(1;9)(q24;q34) translocation. Detailed characterization of the translocation was made using (A) G-banding method, (B) multicolor FISH (Metasystems GmbH, Altlussheim, Germany) and (C, D) two different FISH probes. In panel (C) red signals show hybridization of 1p36 specific probe and green signals binding of 1q25 specific probe with chromosome 9 painting. In panel (D), *BCR-ABL1* ES dual color extra signal translocation probe (Vysis®, Abbott Laboratories, IL, USA) was used showing two normal *BCR* signals from chromosome 22, but three *ABL1* signals. No *BCR-ABL1* fusion signal was detected either with FISH or PCR analysis with different primers for various *BCR-ABL1* transcripts.

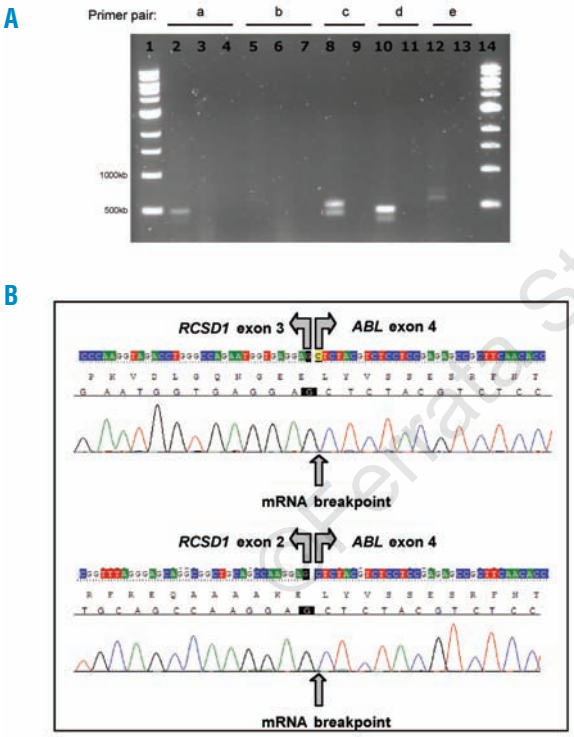

Figure 2. RCSD1-ABL1 fusion gene resulting from a t(1;9)(q24;q34) translocation. Panel (A) shows an agarose gel of PCR products carried out with different *RCSD1-ABL1* primer sets (a-e) (primer pairs aF + bR; aF + cR; bF + aR; bF + bR; bF + cR, respectively). Primers for *RCSD1* were: forward; RCSD1-aF 5'-CCTGAAGGACATGGAGGAAA-3'; RCSD1-bF 5'-CAGAGACCAATGCCAATGTG-3'; binding to exons 1-2. Primers for ABL1 were: reverse; ABL1-aR 5'-CTGCACCAGGTTAGGGTGTT-3', ABL1-bR 5'-TGGTGTCCTCCTTCAAGGTC-3' and ABL1-cR 5'- TCTGAGTGGCCATGTACAGC-3'; binding to exons 4-6. Lanes 1 and 14: DNA ladder; lanes 2, 5, 8, 10 and 12: PCR products of the patient cDNA; lanes 3 and 6: negative control cDNA; lanes 4, 7, 9, 11 and 13: no template control. Two PCR products were visible corresponding to sequencing results shown in panel (B). In the longer PCR product the fusion gene consisted of the three first exons of *RCSD1* gene and in the shorter product lower band only the two first exons were included. In both cases, the *RCSD1* fused to the exon 4 of the *ABL1* gene. The same fusion transcripts were detected on lanes 2 and 12 as in lanes 8 and 10 but the alternatively spliced forms were less efficiently amplified. No PCR products were observed in control reactions with normal cDNA as a template.

dasatinib 70 mg twice daily combined with one cycle of high-dose chemotherapy. The patient successfully re-entered in CCyR, which has been maintained with dasatinib monotherapy for more than nine months.

At the time of diagnosis, a clonal karyotype, 46,XY,t(1;9)(q24;q34)[5]/46,XY[3], was detected in 60% of BM cells (Figure 1A). No other clonal abnormalities were detected. The reciprocal nature of the t(1;9) aberration was confirmed by multicolor FISH (Figure 1B) and refined with FISH probes specific for chromosome 9 and 1p36 and 1q25 regions (Figure 1C). FISH for *BCR-ABL1* gene fusion showed 2 normal BCR signals from chromosome 22, but a split signal of the *ABL1* probe (Figure 1D), which referred to a translocation between *ABL1* and an unknown gene located in chromosome 1 centromeric to 1q25 region.

Based on a previous publication by De Braekeleer *et al.*,[2] involvement of the *RCSD1* gene located in the long arm of chromosome 1 was suspected. Primers covering the kinase domain of *ABL1* and most of the exons of *RCSD1* were designed. PCR analysis from leukemic cells showed 2 positive products of slightly different molecular weights (Figure 2A). Sequencing revealed that the longer PCR product consisted of the first 3 exons of *RCSD1* fused to *ABL1* gene starting from exon 4 extending to the kinase domain coding region (Figure 2B). The

shorter PCR product consisted of the first 2 exons of *RCSD1* fused similarly to exon 4 of *ABL1* (Figure 2B). These two different fusion transcripts were likely caused by alternative splicing of the fusion gene. The predicted oncogenic product of *RCSD1-ABL1* is in-frame and encodes the tyrosine kinase domain of ABL.

Most previously described fusion genes involving *ABL1* (*BCR, ETV6, EML1, NUP214*) fuse with exon 2 of *ABL1*.[3-5] However, similar to *RCSD1-ABL1*, a novel fusion partner (*SFPQ*) was recently reported to fuse to exon 4 of *ABL1* in a pre-B-ALL patient with a t(1;9)(p34;q34) translocation.[6] The fusion protein resulting from *ABL1* exon 4 fusion lacks the amino acids W127-K183 to form intact SH2 domain.[6] Murine experiments have shown that BCR-ABL constructs with inactivated SH2 domain are able to induce B-ALL-like disease,[7] which further supports the central role of *RCSD1-ABL1* in the development of leukemia in our patient. However, additional mechanisms may also exist, as the *RCSD1* gene encodes a protein kinase substrate CapZIP which interacts with the actin capping protein CapZ. This may influence the cytoskeleton regulation and/or migration of the cells.[8]

In Ph$^+$ALL dasatinib is a promising novel treatment option.[1] Our case report describes that TKIs may have clinical efficacy in other types of B-ALL as well. Furthermore, the clinical activity of dasatinib was recently described in a T-ALL patient carrying a *NUP214-ABL1* fusion.[9] Although these fusion genes may occur rarely, the therapeutic implications warrant that they be searched for in acute leukemia. The screen for such uncommon, but clinically significant fusion transcripts should be included in multiplex PCR panels used in the routine diagnostic work-up. Our data further underline the importance of dividing hematologic malignancies into molecularly defined and clinically meaningful disease entities, allowing for a rational and effective selection of optimal treatment modalities for each patient.[10]

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Advanced Philadelphia chromosome positive acute lymphoblastic leukemia patients relapsed after treatment with tyrosine-kinase inhibitors: successful response to clofarabine and cyclophosphamide

Philadelphia chromosome positive acute lymphoblastic leukemia (Ph$^+$ ALL) includes at least one-quarter of adults with ALL. Treatment with tyrosine-kinase inhibitors (TKIs), with or without chemotherapy, today represents the most appealing management both in terms of complete remission (CR) and disease-free survival (DFS), and towards providing eligible patients with a *bridge* to hemopoietic stem cell transplantation (HSCT).[1-4] However, relapsed Ph$^+$ ALL is still regarded as an almost incurable disease. Clofarabine, a second generation deoxyadenosine analog, has demonstrated significant activity in children and adults with refractory lymphoid and myeloid leukemia in early clinical trials.[5] To improve its single-agent antileukemic activity different clofarabine combinations are being studied.[6] With the clofarabine-