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Letter
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BCR-ABL1 Positive B-ALL Can Undergo T-cell Lineage Shift to Become CD19 Negative T-ALL

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Lineage shifts in acute lymphoblastic leukemia (ALL) are uncommon and if they occur usually involve a switch from lymphoid to myeloid lineage.[1–4] Here we report the observation of a B- to T-cell switch in a *BCR-ABL1* positive B-ALL that was engrafted in immune deficient NOD.*Cg-Prkdc*^{scid}*Il2rg*^{tm1Wjl}/SzJ (NSG) mice. We studied the engraftment of 4 *BCR-ABL1* positive B-ALL cases (Table 1), and observed the unexpected appearance of a *BCR-ABL1* positive T-ALL for 1 of the 4 cases (XC49). These data indicate that a switch from B-ALL to T-ALL is possible, and that this could be a novel mechanism to develop resistance against anti-CD19 directed therapy in some B-ALL cases.

Indeed, with the introduction of CD19-directed immunotherapy for the treatment of B-cell malignancies, clinicians were suddenly confronted with previously unobserved tumor escape mechanisms. More and more patients present themselves at relapse with a downregulation or alteration of the CD19 surface antigen on their malignant cells or with an acquired extramedullary invasion.[5–12] In a recent study, Nagel et al described an alternative escape route for malignant B-ALL cells after receiving anti-CD19 directed therapy. They presented 2 cases of *BCR-ABL1* positive B-ALL who experienced a CD19-negative myeloid lineage relapse after blinatumomab treatment. Using fluorescence in situ hybridization, the authors confirmed the presence of the *BCR-ABL1* fusion in the hematopoietic stem cell (HSC) compartment of both patients. Investigation of an additional 25 *BCR-ABL1* positive B-ALL cases revealed that up to 40% of the patients had HSC involvement. Interestingly, HSCs were more often involved in patients carrying the major *BCR-ABL1* transcript encoding P210$^{BCR-ABL1}$ than patients carrying minor *BCR-ABL1* transcripts encoding P190$^{BCR-ABL1}$.[13]

Here, we describe a B to T lineage shift in a patient-derived xenograft (PDX) model from a childhood *BCR-ABL1* positive, CD19-positive B-ALL (case XC49, Table 1). This 3-year-old female patient was admitted to the hospital with fever and musculoskeletal pain. Clinical and laboratory investigations revealed hepatosplenomegaly with signs of anemia and moderate thrombocytopenia in the peripheral blood. White blood cell counts were elevated, up to 60,000 cells/mm^3, with 58% blast cells and a bone marrow smear revealed a blast cell count of 80%. Karyotyping and MLPA investigation identified a t(9;22)(q34;q11) resulting in the *BCR-ABL1* fusion transcript e1a2 (P190). Karyotyping also detected hyperdiploidy of chromosomes X, 4, 9, 10, 14, 21, and 22. IgH and IgK were monoclonal, as was the T-cell receptor gamma, which was consistent with a monoclonal B-cell proliferation with illegitimate T-cell receptor rearrangement. The patient was diagnosed with high risk B-ALL, exceptionally carrying both a P190$^{BCR-ABL1}$ fusion transcript and a hyperdiploid phenotype. Treatment was given according to the EsPhALL protocol and imatinib was associated during induction chemotherapy. Minimal residual disease investigations were negative after induction therapy, but unfortunately the child deceased 10 months after initial diagnosis, due to treatment side effects.

Bone marrow and peripheral blood samples were collected both at diagnosis and after induction when the patient was in complete remission, according to protocol S57176 approved by the Ethical Committee of the University Hospital of Leuven. Written informed consent was obtained. Mononuclear cells were isolated from the samples using Ficoll-Paque and used for RNA and DNA extraction. Whole genome sequencing (WGS) of the patient sample at diagnosis compared to remission confirmed the presence of a P190$^{BCR-ABL1}$ fusion transcript and the hyperdiploidy of chromosomes X, 4, 9, 10, 14, 21 and part of chromosome 22 (Fig. 1A). Flow cytometry of the cells revealed a

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Table 1

Patient Characteristics

Patient Code	Gender	Age, y	WBC	Blast Count % (PB)	Blast Count % (BM)	Karyotype	FISH	DNA Index	Immune Phenotype	Xenograft
XA19	Male	15	61,500	91	97	44,XY,del(2)(q23q36),−7, −8,der(9)t(8;9)(q11;p13), add(14)(q31)/45,SL, +MAR1	t(9;22)(q34;q11)	1.00	Positive for CD10, CD19, CD20, CD22, CD34, cyCD79a, cylgM, TdT, HLA-DR; negative for slgKappa/Lambda	n=3; 3x B-ALL
XB40	Female	7	22,980	46	92	46,XX,t(9;22)(q34;q11)/53,XX, +X,+4,+6,t(9;22)(q34;q11), +14,+17, +21, +der(22)t(9;22)	t(9;22)(q34;q11)	1.12	Positive for CD10, CD19, CD20, CD22, CD34, cyCD79a, cylgM, TdT, HLA-DR; negative for slgKappa/Lambda	n=2; 2x B-ALL
XC49	Female	3	58,770	58	80	46,XX,t(9;22)(q34;q11)[1]/54, sl, +X,+4,+9,+10,+14, +21,+22,+der(22)t(9; 22)[1]/46,XX[3]	t(9;22)(q34;q11)	1.18	Positive for CD10, CD19, CD20 (partially), CD22, CD34, cyCD79a, cylgM, TdT, HLA-DR; negative for cyCD3, sCD3, CD117, slgKappa/Lambda	n=7; 4x B-ALL; 3x T-ALL
XC51	Male	5	1330	4	86	46,XY	t(9;22)(q34;q11)	1.00	Positive for CD10, CD19, CD22, CD34, cyCD79a, cylgM, TdT, HLA-DR; negative for CD20, slgKappa/Lambda	n=4; 4x B-ALL

Xenograft data indicate the number of NSG mice obtained with B-ALL or T-ALL, only in case XC49 we obtained both B-ALL and T-ALL xenografts.
BM=bone marrow, PB=peripheral blood, WBC=white blood cell count in the peripheral blood (cells/mm^3).

CD19-positive, CD34-positive phenotype of the leukemia cells (Fig. 1B).

Diagnostic mononuclear cells were injected into the tail vein of seven 6-to-12-week old NSG mice for further leukemic expansion and analysis. Blood samples were taken once every 2 weeks and measured on a Vet ABC Hematology Analyzer (SCIL). Leukemic engraftment was determined with flow cytometry (FACS Canto II, BD, San Jose, CA) using antihuman CD19 (FITC, eBioscience, Waltham, MA) as a marker for the leukemic cells. Of the 7 mice that were injected, 3 were sacrificed after 41 to 77 days due to ethical end points (without signs of CD19-positive cells), while the remaining 4 animals showed successful engraftment of the CD19-positive B-ALL and were sacrificed 10 to 20 days later (Fig. 1C). Staining of the splenic and bone marrow cells revealed that the first 3 animals developed a weak CD34-positive, CD19-negative leukemia and that these leukemic cells were positive for CD2, CD5, and cytoplasmic CD3. The expression of CD4, CD8, and CD7 varied between the xenografts. Engraftment was lower (23–32% based on intracellular human CD3) than for the other 4 xenografted animals, which showed invasion of their spleens with >80% CD19-positive, CD34-positive leukemia cells, similar to the B-ALL of the patient (Fig. 1D–G). Leukemic cells from both the T- and B-ALL PDX model were transplantable into secondary recipients (data not shown).

We next investigated the presence of the *BCR-ABL1* fusion in cDNA and DNA derived from bone marrow and spleen cells of the xenografted animals. The *BCR-ABL1* fusion was detected in all animals irrespective of B-ALL or T-ALL immunophenotype. The exact same *BCR* and *ABL1* breakpoints (determined by whole exome sequencing, Fig. 1A) were detected at genomic level in B- and T-ALL, confirming that the T-ALL originated from the same leukemia stem cells (Fig. 1H).

In 1999, Winter et al[14] reported an unusual pre-B ALL case with b3a2 (P210) and e1a2 (P190) *BCR-ABL1* fusion transcripts that relapsed as chronic myeloid leukemia carrying only the less differentiated b3a2 (P210) clone. This case report already alluded on the existence of precursor cells with both myeloid and lymphoid potential involved in *BCR-ABL1* leukemia development. More recently, Nagel et al reported that up to 40% of the *BCR-ABL1* positive B-ALL patients had HSC involvement, and linked that to the possibility for B to myeloid leukemia shifts during anti-CD19 directed therapy. Here, we describe yet another possible mechanism for escape to anti-CD19 therapy by switching from B- to T-ALL. Our data provide additional evidence for the existence of CD19-negative, *BCR-ABL1* positive leukemia stem cells in B-ALL.

Our patient carried a minor *BCR-ABL1* transcript $P190^{BCR\text{-}ABL1}$, which is, according to Castor et al,[15] precluded to the B-cell progenitor stage. Although Nagel et al also primarily found HSC-involvement in patients carrying major *BCR-ABL1* transcript encoding $P210^{BCR\text{-}ABL1}$, they did detect HSC-involvement in 3 out of 12 patients with minor $P190^{BCR\text{-}ABL1}$ transcripts.[13]

While *BCR-ABL1* translocations can occur in T-ALL, they are very rare and the NUP214-ABL1 fusion is more common in T-ALL.[16–18] Our data however indicate that the increasing use of CD19-directed therapies may guide leukemic cells to more inventive escape mechanisms, not only by relapsing as CD19-negative, *BCR-ABL1* positive myeloid leukemia, but possibly also as CD19-negative, *BCR-ABL1* positive T-ALL.

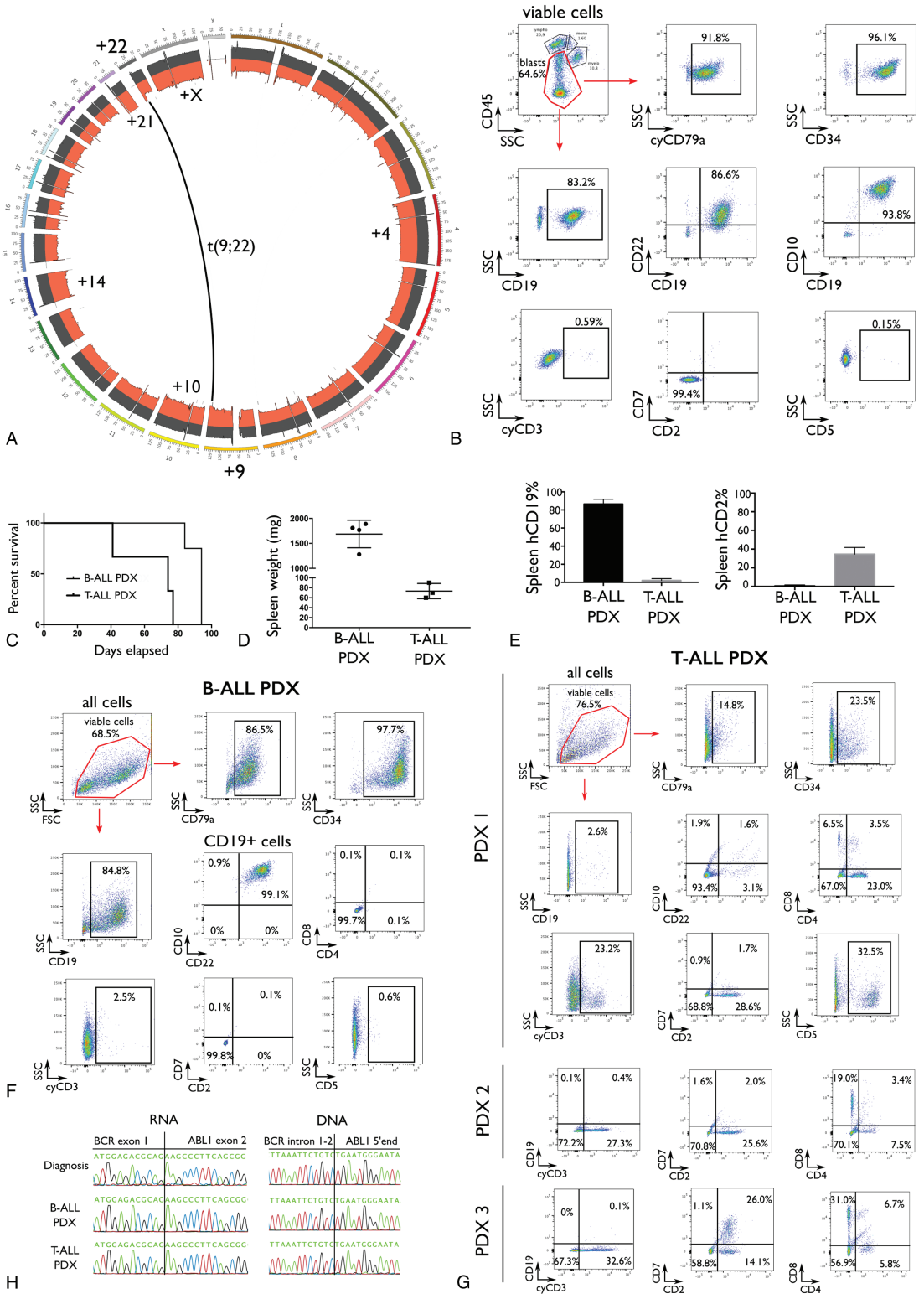

Figure 1. B- to T-cell lineage shift after injection of the *BCR-ABL1* positive B-ALL (XC49) into immune deficient mice. (A) Circos-plot of the primary patient sample (XC49) confirming the presence of a t(9;22) and hyperdiploidy of chromosomes 4, 9, 10, 14, 21, X and partial chromosome 22. (B) Flow cytometric staining of the diagnostic patient sample (XC49). All plots are gated on the leukemic blast cells unless mentioned otherwise above the graph. (C) Survival curve comparing survival times for B-ALL and T-ALL developing xenografts after injection of the primary *BCR-ABL1* positive sample (XC49). (D) Plot presenting the spleen weights of B-ALL and T-ALL xenografts. (E) Bar charts showing the percentage of human CD19- and CD2-positive leukemic cells present in the spleens of B-ALL and T-ALL xenografts. (F) Flow cytometric staining of leukemic cells present in the spleen of a representative B-ALL xenograft. Plots are gated on viable cells unless mentioned otherwise above the graph. (G) Flow cytometric staining of leukemic cells present in the spleens of the T-ALL xenografts. Full staining is provided for one of the xenografted animals (PDX1), additional plots are provided for the other 2 animals (PDX2 and 3), illustrating the differential expression in CD7, CD4, and CD8. Plots are gated on viable cells unless mentioned otherwise above the graph. (H) Sanger sequencing confirming the presence of the *BCR-ABL1* fusion on cDNA and DNA derived from leukemic cells at diagnosis and from B-ALL and T-ALL xenografts.

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