Multilineage involvement in ABL-class fusion–positive pediatric B-cell acute lymphoblastic leukemia: CML-like biology

We describe a potential new subtype of B-cell acute lymphoblastic leukemia (B-ALL), characterized by the presence of an ABL-class fusion in lymphoblasts and seemingly normal hematopoietic lineages. The presence of the fusion gene in multiple hematopoietic lineages may have implications for diagnosis, prognosis, and therapeutic approaches.

Our patient was diagnosed with B-ALL in November 2015 at two years of age and treated according to the Dutch Childhood Oncology Group (DCOG)-ALL11 protocol. The parents gave written informed consent in accordance with the Declaration of Helsinki to use the data for research purposes. The Máxima Biobank research protocol was evaluated as a non-interventional study by the Board of the Medical Ethics Committee NedMec (Utrecht, the Netherlands) and the Biobank and Data Access Committee approved this project. Because of M2 marrow and persisting high levels (50%) of minimal residual disease (MRD) at end of induction (EOI) as detected by RQ-PCR analysis of rearranged immunoglobulin (IG) and/or T-cell receptor (TR) genes, he was treated with high-risk (HR) chemotherapy courses followed by maintenance therapy. He became MRD-negative after one HR course and remained MRD-negative for these IG/TR targets during the rest of his treatment. One year after cessation of therapy, in November 2018 at the age of five years, he was diagnosed with medullary relapsed B-ALL. Remarkably, PCR analysis using the original IG/TR RQ-PCR targets was negative. Fluorescent *in situ* hybridization (FISH) analysis demonstrated a 5q32 *PDGFRB* rearrangement, thereby defining the ALL subtype as ABL-class fusion ALL. ABL-class fusions are detected in approximately 3% of children with ALL and are defined as fusions involving one of the tyrosine kinase genes, *ABL1, ABL2, PDGFRB,* or *CSF1R*, fused to various partner genes, excluding the sentinel *BCR*::*ABL1* fusion. Subsequent RNA sequencing identified a *CCDC88C*::*PDGFRB* fusion caused by a t(1;14;5)(p22;q32;q32) translocation. Genomic capture located the breakpoints in intron 12 of *CCDC88C* (Hg38 chr14: 91,323,275) and intron 10 of *PDGFRB* (Hg38 chr5: 150,129,441) fusing the HOOK domain encoded by *CCDC88C* to the protein tyrosine kinase domain encoded by *PDGFRB*.[1] Based on this finding, archived viable cells from initial diagnosis were re-analyzed and the three-break rearrangement indicative for the *CCDC88C*::*PDGFRB* fusion was

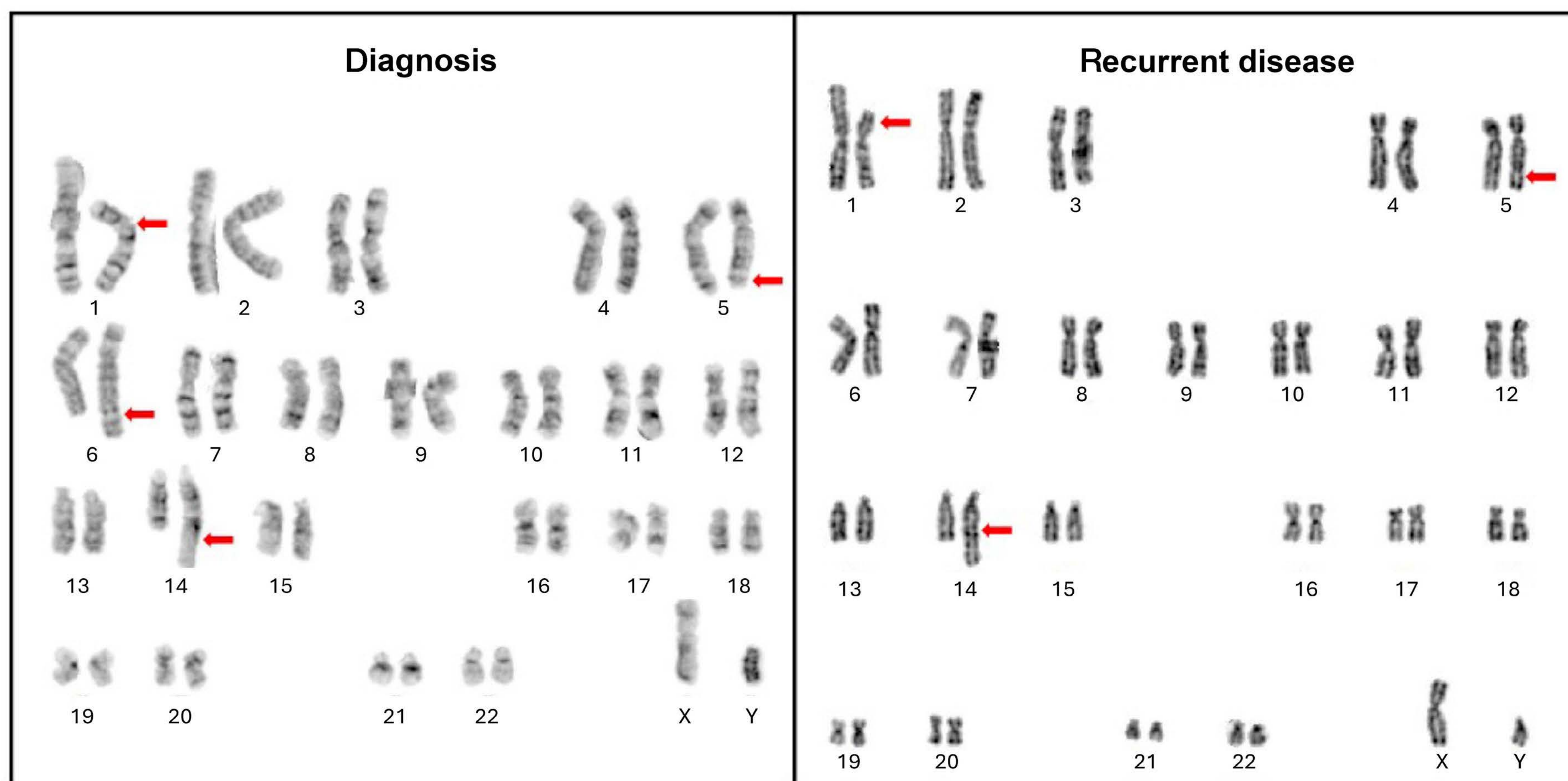


Figure 1. Karyograms by GTG-banding of bone marrow at first diagnosis and at recurrent disease. (A) Karyogram at diagnosis in 2015, karyotype 46,XY,t(1;14)(p22;q24),del(5)(q32q34). (B) Karyogram at recurrent disease in 2018, karyotype 46,XY,t(1;14;5)(p22;q32;q32). Red arrows point to affected chromosomes.

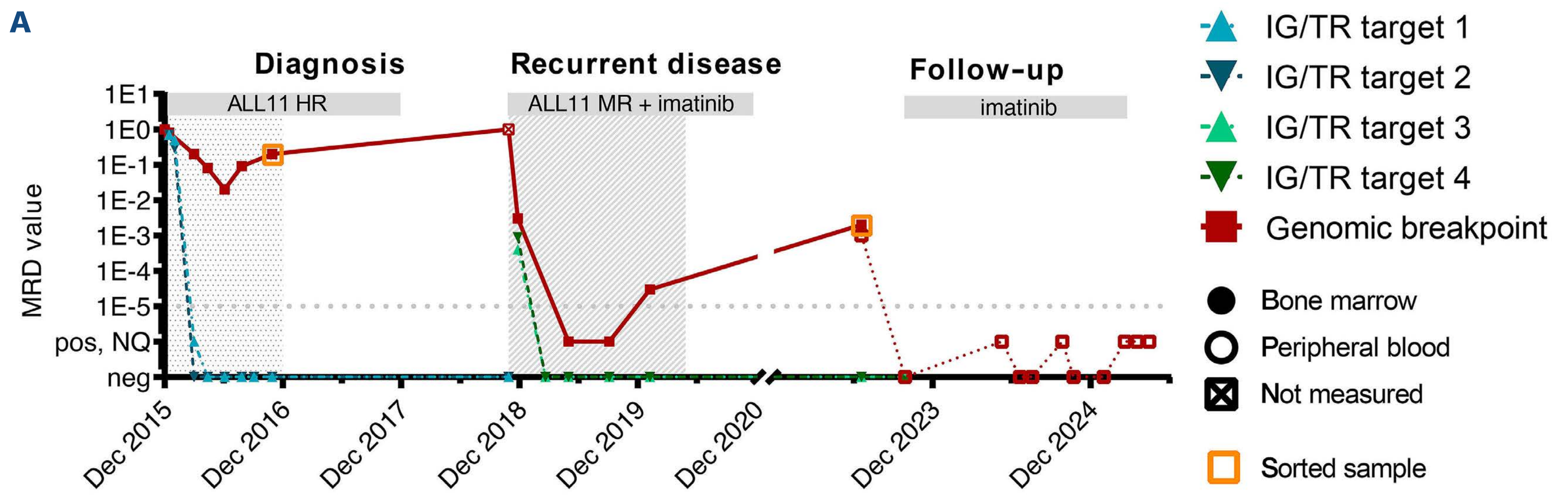
confirmed to be present (Figure 1). Eosinophilia, which has been associated with *PDGFRB*-rearranged leukemias, was absent. The patient was treated according to the DCOG-ALL11 medium risk group schedule with addition of imatinib. This time, the patient responded well to induction treatment, with morphologically complete remission (CR) at EOI. MRD based on newly identified IG/TR targets was 0.09% at EOI and negative at end of consolidation. Further treatment according to DCOG-ALL11 with additional imatinib for two years was uneventful.

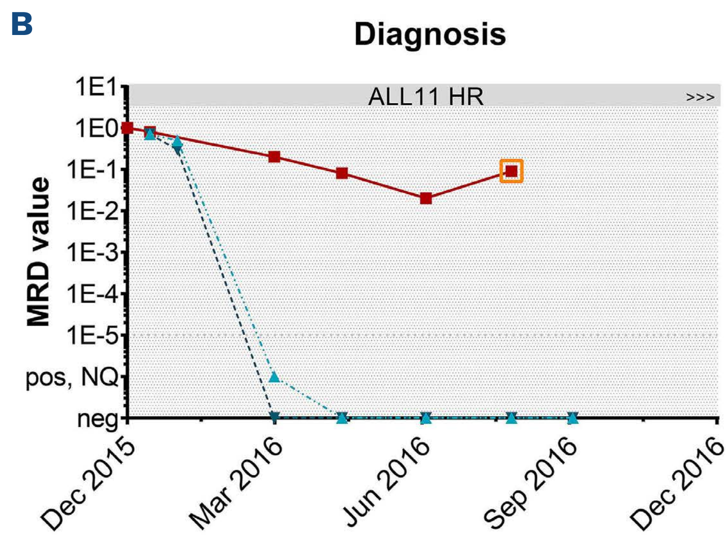
We developed an MRD assay to monitor the fusion gene using the genomic breakpoint, as reported previously in a larger MRD study which included limited samples from the current patient.[1] The quantitative range of the genomic breakpoint RQ-PCR and the IG/TR RQ-PCR of the targets used for the clinically reported MRD at first diagnosis and at disease recurrence were all 10^{-5} with sensitivities of 10^{-5}. We found discordant MRD levels at all timepoints of initial treatment, with ABL-class fusion MRD-positivity at the level of 2-20%, despite morphological remission and IG/TR MRD-negativity. We extended the MRD comparison over the disease course (Figure 2A-C). At what was initially considered as relapse (November 2018), the patient-specific ABL-class fusion MRD was high after re-induction (0.05%) and decreased to ranges between 'positive, not quantifiable' (pos-NQ) and 0.001% during treatment with DCOG-ALL11 plus imatinib. In contrast, IG/TR-based MRD using the newly identified targets was negative from end of consolidation onwards. Given the retrospective discrepant MRD values both at initial diagnosis and at disease recurrence, a bone marrow aspiration was performed to reassess potential persistence of molecular MRD in June 2023, 2.5 years after the end of maintenance and imatinib treatment. The patient was in good clinical condition, with normal blood cell counts. Bone marrow and blood showed complete remission, MRD-negativity with IG/TR PCR, but MRD-positivity for ABL-class fusion PCR of 0.1% in blood and 0.2% in bone marrow. Monotherapy with imatinib was started in June 2023, resulting in decreased *CCDC88C*::*PDGFRB* fusion MRD levels ranging from pos-NQ to negative up till February 2025. Imatinib was discontinued in February 2025 based on growth inhibition (standard deviation score [SDS] length for age from -1.3 SDS to -1.8 SDS), which was also observed during initial imatinib treatment from 2018 until 2020 (from -0.8 SDS to -1.9 SDS). After stopping imatinib, MRD levels for the *PDGFRB* fusion were stable (pos-NQ) up to last follow-up in April 2025.

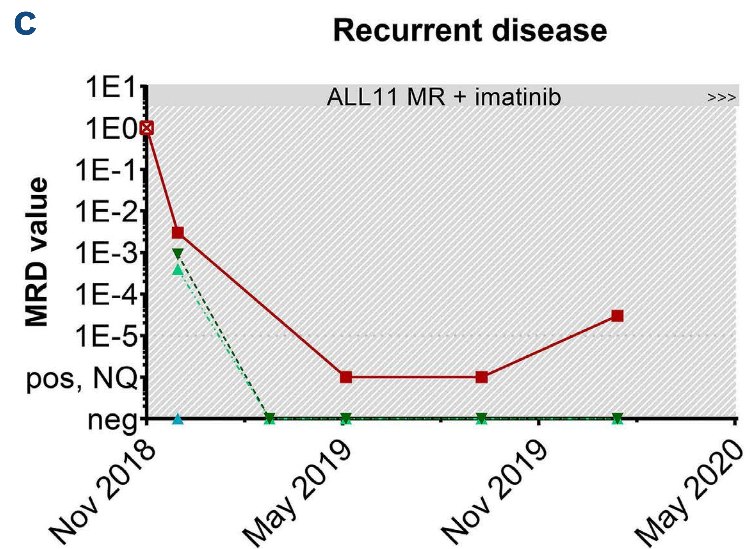
The discrepant IG/TR and ABL-class fusion PCR results suggested presence of the fusion in hematopoietic cells other than leukemic B-lymphoblasts. Therefore, we sorted the myeloid and lymphoid fractions of cryopreserved bone marrow obtained in first complete remission (CR1) and the bone marrow sample after completion of treatment for disease recurrence (CR2). Analyses of CR1 and CR2 by FISH and RQ-PCR, respectively, showed presence of the fusion gene in both the myeloid and lymphoid cells at similar percentages in both fractions: sample taken in CR1 20% in myeloid fraction, 10% in lymphoid fraction; sample taken in CR2 0.09% in myeloid fraction, pos-NQ in lymphoid fraction (Figure 2D-F). These results confirmed that the ABL-class fusion gene was not only present in the leukemic blasts of the patient, but also in multiple seemingly normal hematopoietic cell lineages. Whole exome analysis showed no common, but only different, pathogenic secondary cytogenetic abnormalities, in addition to the *CCDC88C*::*PDGFRB* fusion between the blasts at initial diagnosis and at blast recurrence (Figure 3). The detection of the fusion in myeloid as well as lymphoid hematopoietic cells during remission, and the different secondary lesions and IG/TR rearrangements between initial diagnosis in 2015 and recurrence in 2018, suggest that the fusion occurred before lineage commitment in a hematopoietic stem cell or multipotent progenitor cell. Interestingly, recent RNA-sequencing studies revealed that, at initial diagnosis of *BCR*::*ABL1*-positive ALL, both cases with a lymphoid gene expression signature as well as cases with a multilineage signature were found.[2-4] A preliminary extension of the original classifier[2] to detect multilineage involvement across subtypes other than BCR::ABL1 predicted our patient's diagnostic sample as multilineage (*T. Beder and L. Bastian, personal communication, 2025*). Together, our data suggest that the recurrence was a second leukemic clone developed from a *CCDC88C*::*PDGFRB*-positive multipotent progenitor.

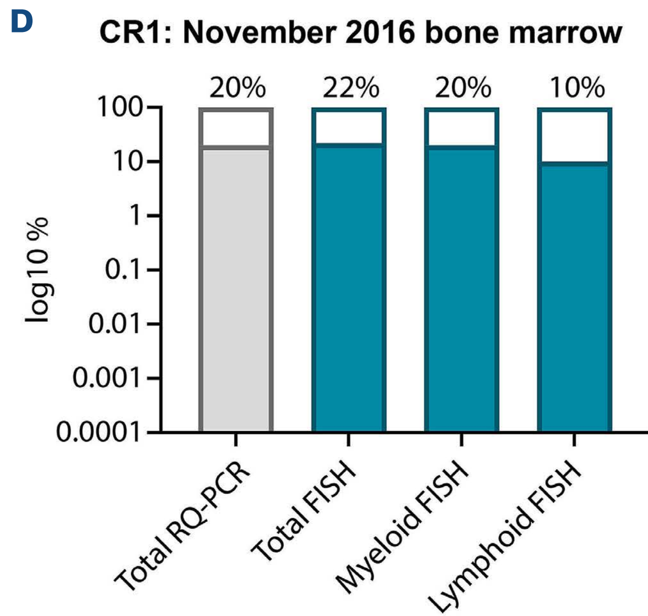
PDGFRB fusions, including *CCDC88C*::*PDGFRB*, have also been reported in pediatric patients diagnosed with myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions (MLN-TK).[5,6] In our patient, no signs of eosinophilia or other myeloproliferative disease were present. The disease in our patient also differed from pediatric patients with *PDGFRB* fusion-positive MLN-TK with lymphoid blast crisis reported earlier,[6-8] as these latter patients presented with T-lymphoblastic lymphoma and eosinophilia and without detectable blasts in blood or bone marrow. In contrast, our patient presented twice with full-blown B-lymphoblastic leukemia. In the absence of any signs of myeloproliferative disease and the low incidence of *de novo* B-lymphoid blast crisis in MLN-TK in children, we propose to classify the disease in our patient as "ABL-class fusion-positive B-ALL with multilineage involvement". This nomenclature is in line with the recent distinction between *BCR*::*ABL1*-positive B-ALL with lymphoid involvement only and *BCR*::*ABL1*-positive B-ALL with multilineage involvement (referred to as chronic myeloid leukemia [CML]-like).[5,9]

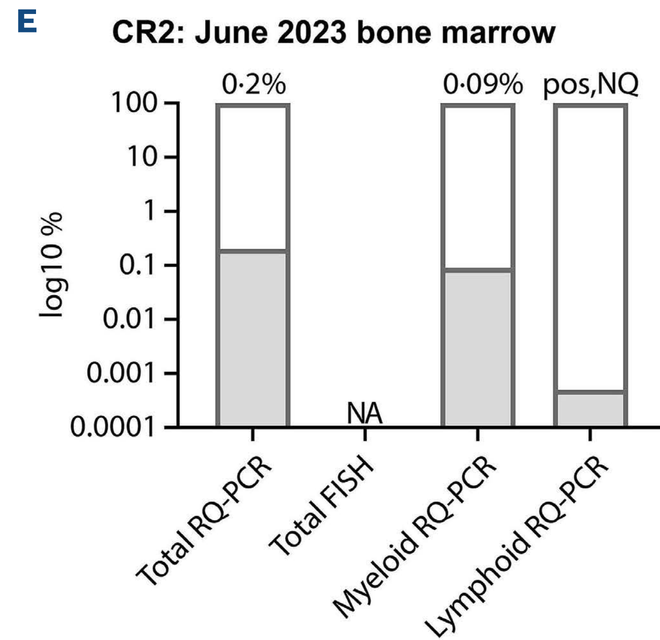
Previous studies comparing MRD for *BCR*::*ABL1* and IG/TR targets revealed residual *BCR*::*ABL1*-positive non-ALL cells in 25-40% of studied adult and pediatric ALL cases.[9,10] Based on these findings, the updated international consensus classification divides *BCR*::*ABL1*-positive B-ALL into two biologically distinct subsets: lymphoid only, in which the fusion event occurred in a lymphoid progenitor, and multi-











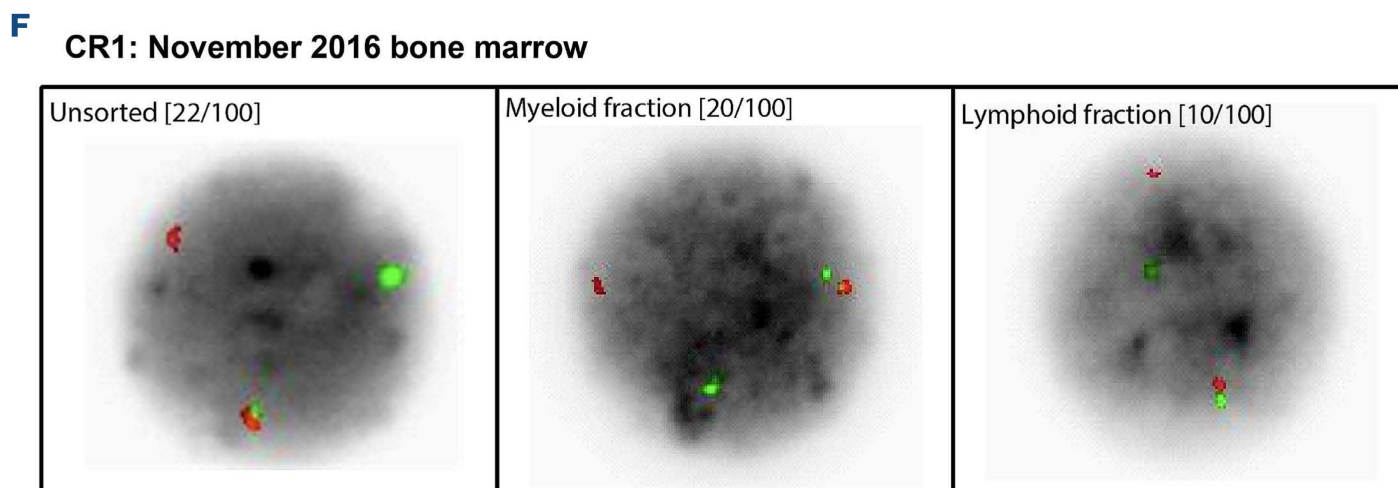


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Figure 2. Minimal residual disease levels on total and sorted myeloid and lymphoid lineages at different time points during the care trajectory. (A-C) Comparison between clinically reported minimal residual disease (MRD) based on IG/TR and genomic breakpoint RQ-PCR MRD during the complete care trajectory of diagnosis (enlarged in panel B), recurrent disease (enlarged in panel C), and follow up. The treatments are indicated in gray bars on top of the MRD plot. At presentation of recurrent disease, MRD was not measured and the depicted square with the cross shows the lymphoid blast percentage in the bone marrow by morphology (64%). MRD levels based on IG/TR RQ-PCR (triangles) were compared with genomic breakpoint PCR (squares) in the bone marrow (solid fill, solid line) or blood (no fill, dotted line). Time points of the two samples used to sort myeloid and lymphoid lineages are highlighted by an orange square. (D and E) Viable frozen cells from November 2016 (D) and June 2023 (E) were FACS-sorted to myeloid ($CD13^+/CD33^+$) and lymphoid (size-based, $CD13^-/CD33^-$ or $CD3^+/CD19^+$) fractions using a Sony SH800S Cell Sorter. Purity by flow cytometry was >97% for myeloid and >99% for lymphoid fractions. *CCDC88C*::*PDGFRB* fusion presence in the total populations and lymphoid and myeloid fractions were measured by genomic breakpoint RQ-PCR (gray bars) and break-apart FISH using *PDGFRB* probes (blue bars). (F) Representative interphase nuclei after fluorescent *in situ* hybridization with *PDGFRB* break-apart probes on total and sorted cells from bone marrow aspirate of November 2016. Overlying dots indicate the intact gene, while separated red and green dots indicate *PDGFRB* disruption. Numbers between square brackets indicate the number of nuclei counted with break-apart pattern in 100 total nuclei counted. Probes were from Vysis LSI PDGFRB Break Apart FISH Probe Kit (Abbott).

lineage, in which the fusion event occurred in a multipotent progenitor and is also called CML-like.[5] Patients with multilineage *BCR*::*ABL1*-positive ALL had similar disease-free survival and only IG/TR MRD was demonstrated to have prognostic value.[2,10-12] However, *BCR*::*ABL1* instead of IG/TR MRD monitoring after end of treatment, at 3-6 monthly intervals, was recommended for early detection of disease reoccurrence due to potential loss of IG/TR targets.[12] In line with this recommendation, we have continued monitoring the *PDGFRB* genomic breakpoint in our patient.

In patients with CML, long-lasting treatment with imatinib is recommended to reach long-term remission.[13] After discontinuation of imatinib in patients without a sustained deep molecular response, *BCR*::*ABL1*-positive cells can recur.[13]

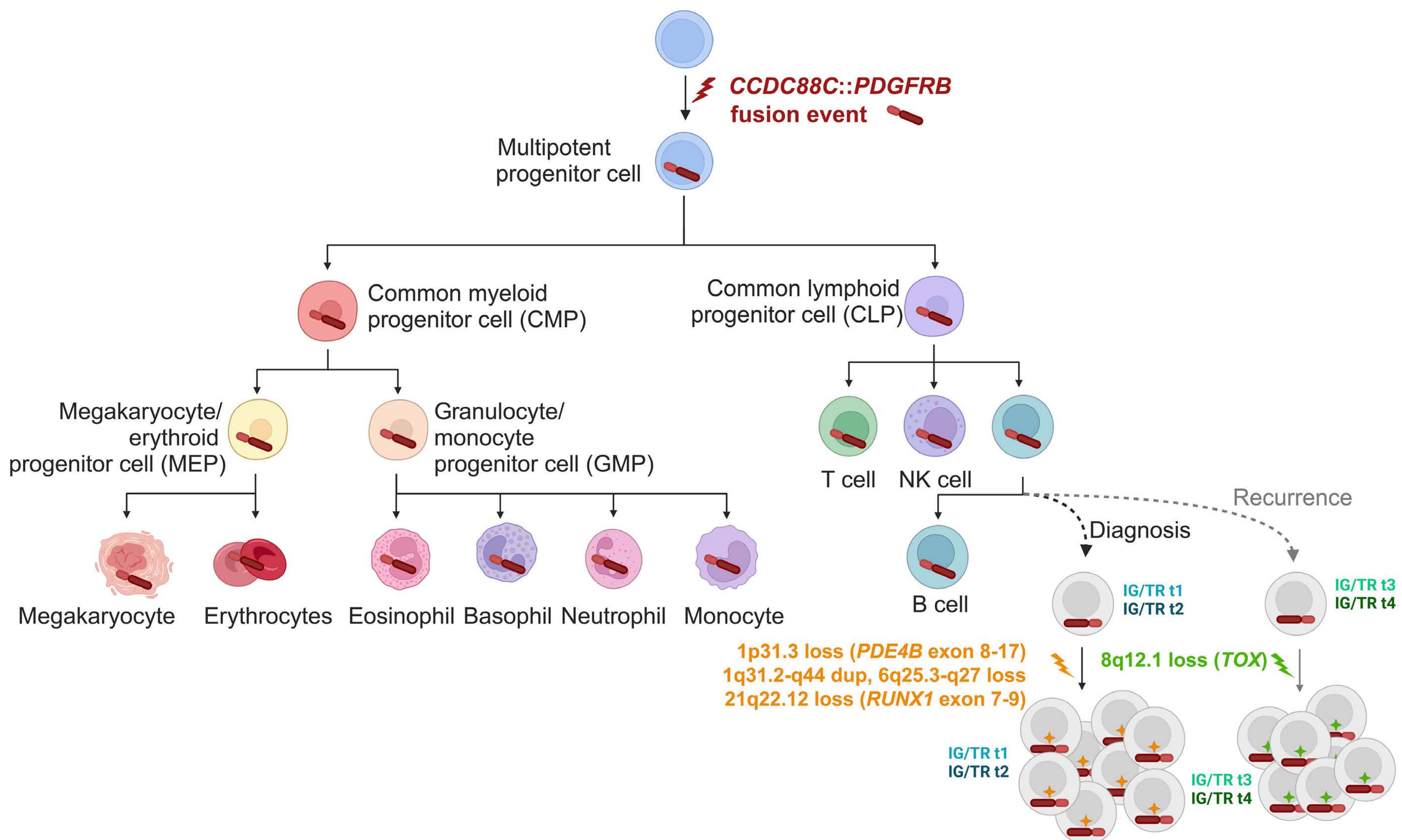

Figure 3. Schematic overview of hematopoietic development in the case of an ABL-class fusion gene in a multipotent progenitor cell. A multipotent progenitor cell acquired the *CCDC88C*::*PDGFRB* fusion, which resulted in the presence of the fusion gene in all hematopoietic lineages (myeloid and lymphoid). In an early stage of development, B cells acquired additional cytogenetic abnormalities detected by whole exome sequencing. The leukemia at diagnosis presented with a 1p31.3 loss resulting in a deletion of exon 8-17 of *PDE4B* (NM_002600), a 1p31.3 gain, a 6q25.3-q27 loss, and a 21q22.12 loss resulting in the deletion of exon 7-9 of *RUNX1* (NM_024007). These aberrations were present in 70% of the cells at first diagnosis and absent at disease recurrence. The leukemia at re-occurrence presented with an 8q12.1 loss resulting in deletion of *TOX* in 75% of the cells, which was absent at first diagnosis. NK: natural killer.

In our patient, in a long follow-up period without imatinib treatment, ABL-class fusion-positive cells recurred at 0.2%, and dropped to below 0.001% after restart of imatinib. This suggests that long-lasting treatment with imatinib or other tyrosine kinase inhibitors[6,7] may be necessary, although it is unknown whether a very low level of fusion persistence would lead to relapse without tyrosine kinase inhibitor continuation.[14] Alternatively, allogeneic hematopoietic stem cell transplantation to irradicate the ABL-class fusion carrying hematopoietic precursor cells may be indicated if treatment with imatinib or other tyrosine kinase inhibitors becomes ineffective. In line with the recommendation of the international pediatric CML expert panel, a pre-emptive approach with close monitoring of ABL-class fusion MRD to guide restart of tyrosine kinase inhibitor treatment may be recommended for ABL-class fusion ALL cases with multilineage involvement.[15]

In conclusion, we identified a new biological entity of ABL-class fusion B-ALL characterized by multilineage involvement which resembles *BCR*::*ABL1*-positive B-ALL with multilineage involvement (often referred to as CML-like type). We recommend MRD monitoring by IG/TR and ABL-class fusion in the early stages of treatment to distinguish from typical ABL-class fusion-positive B-ALL with lymphoid involvement only.

Authors

Inge van Outersterp,[1*] Judith M. Boer,[1*] Edwin Sonneveld,[1] Anja X. de Jong,[1] Stefan Nierkens,[1,2] Vincent H.J. van der Velden,[3] Arjan Buijs,[4] Maaike Luesink,[1] Monique L. den Boer[1,2#] and Peter M. Hoogerbrugge[1#]

[1]Princess Máxima Center for Pediatric Oncology, Utrecht; [2]Center for Translational Immunology, University Medical Center Utrecht, Utrecht; [3]Department of Immunology, Laboratory Medical Immunology, Erasmus MC, University Medical Center Rotterdam, Rotterdam and [4]Department of Genetics, University Medical Center Utrecht, Utrecht, the Netherlands

*IvO and JMB contributed equally as first authors.

#MLdB and PMH contributed equally as senior authors.

Correspondence:
MONIQUE DEN BOER - m.l.denboer@prinsesmaximacentrum.nl

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Contributions
MLdB, PH and IvO conceptualized the study; MLdB, PH, JMB, SN, ML and VHJvdV designed and supervised the study; PH, ES, AXdJ and AB collected clinical and diagnostic data; IvO, JMB, MLdB and PH analyzed and interpreted data, and wrote the first draft of the manuscript. All authors reviewed, revised, and approved the final manuscript.

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Data-sharing statement
For original data, please contact the corresponding author at m.l.denboer@prinsesmaximacentrum.nl.

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