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CASE REPORT

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Successful treatment of T/myeloid mixed-phenotype acute leukemia with the translocation (10;11)(p13;q14) *PICALM/AF10* with 3 + 7 myeloid standard treatment: A case report

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Abstract

The translocation *PICALM/AF10* is described in multilineage diseases. We report a patient with *PICALM/AF10* T/myeloid mixed-phenotype acute leukemia who achieved durable complete remission after AML-like treatment suggesting a myeloid origin.

KEYWORDS

mixed-phenotype acute leukemia, PICALM-AF10, translocation (1011)(p13q14)

1 | INTRODUCTION

Mixed-phenotype leukemia (MPAL) is a rare and high-risk subtype of leukemia accounting for less than 1% of all leukemias. No consensus exists regarding appropriate treatment. We report a patient with *PICALM/AF10* T/myeloid MPAL who reached durable complete molecular remission after acute myeloid leukemia (AML)-like treatment.

Mixed-phenotype acute leukemia is a heterogeneous group of leukemias that is extremely rare, accounting for less than 1% of all acute leukemias.[1] Several hematopoiesis patterns have been proposed to explain this mixed phenotype, but the hematopoiesis is more complex than linear models. We report the case of a young man who developed T/myeloid mixed-phenotype leukemia with extranodal damage and translocation (10;11)(p13;q14) *PICALM/AF10*. The translocation (10;11)(p13; q14) *PICALM/AF10* is described in multilineage blood disease, but the physiopathology of *PICALM/AF10*-mediated leukemia remains unresolved. PICALM (phosphatidylinositol-binding clathrin assembly protein, or CALM) is a ubiquitously expressed protein involved in clathrin-mediated endocytosis and iron homeostasis.[2] AF10 is a transcriptional factor and one of the fusion partners of MLL. The patient achieved complete and durable remission with an AML regimen (3 + 7), followed by HLA-matched unrelated allogeneic stem cell transplantation. Because of limited available data, no gold standard of care exists. An acute lymphoblastic leukemia (ALL)-like regimen followed by allogeneic

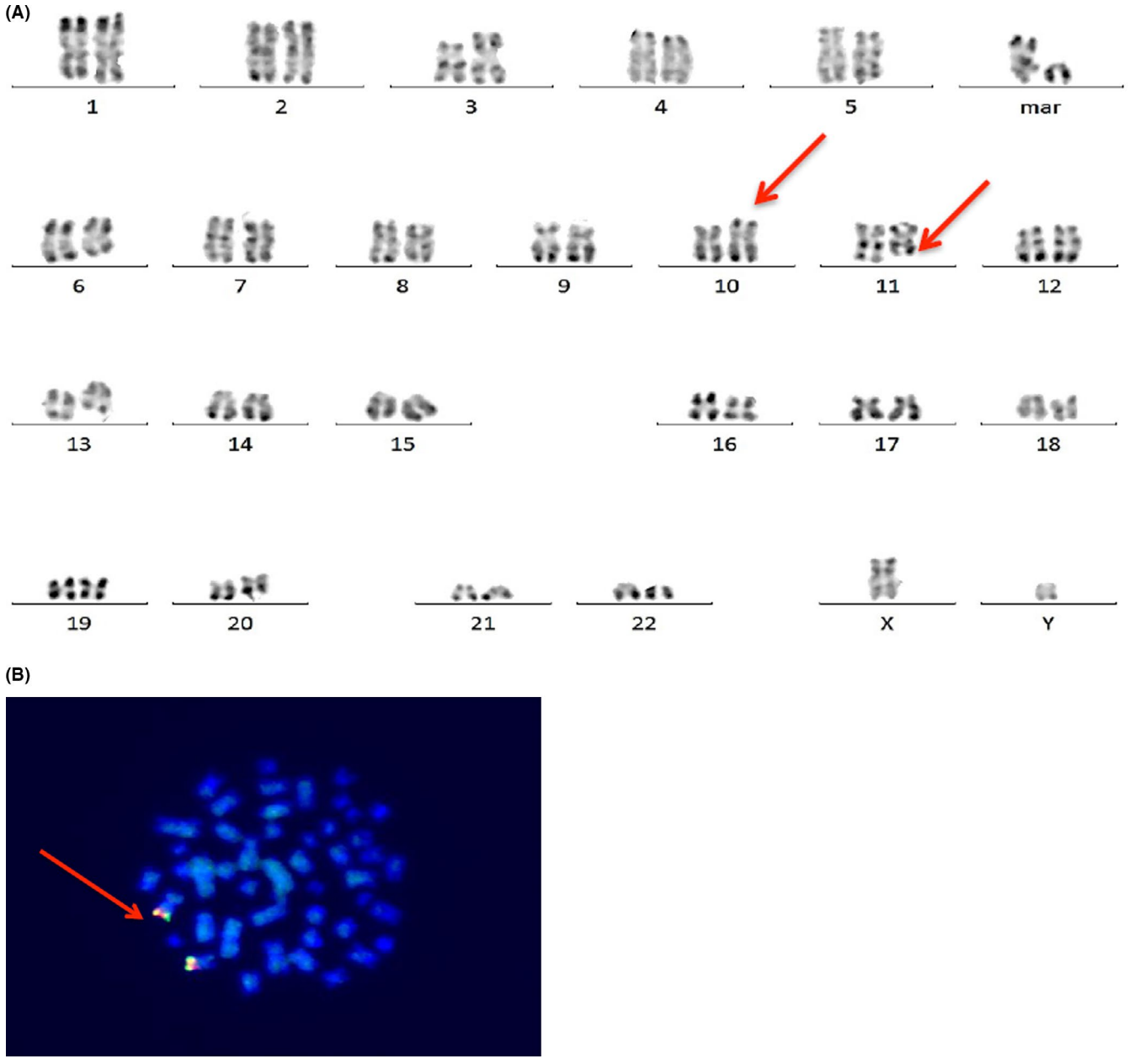

FIGURE 1 (A) Karyotype of our patient showing translocation (10;11)(p13;q14). (B) FISH with MLL break-apart probes. An 87-kb probe, labeled in red, covering a region telomeric to the MLL (KMT2A) gene including the marker D11S3207 and a green probe covering a 170-kb region centromeric to the MLL gene spanning the CD3G and UBE4A genes. The red and green probes are both translocated on 10p, indicating that MLL is not rearranged

stem cell transplantation is currently advised[3]; however, the overall survival of MPAL is poorer than that of ALL (B or T) or AML.

2 | MEDICAL HISTORY

A 33-year-old man without a relevant medical history sought medical advice because of the rapid appearance of unilateral palpebral ptosis in February 2019. He described night sweats contrasting with overall good physical condition. No tumor syndrome was found other than tumefaction of the medial canthus of the left eye.

Blood tests showed isolated hyper leukocytosis (at 17G/L), with 80% of blast cells, no cytopenia, disseminated intravascular coagulation (DIC) or tumor lysis syndrome (TLS). Bone marrow aspiration revealed acute myeloblastic leukemia without maturation, including most cells expressing CD34 (98.5%), CD38 (90.5%), and myeloid (CD33 and CD117 and intracytoplasmic myeloperoxidase) and lymphoid (CD7 and CD3) differentiation antigens. The medullary karyotype was complex and displayed the unbalanced

translocation (10;11)(p13;q14) *PICALM/AF10,* confirmed by FISH (Figure 1) and multiplexed targeted sequencing of recurrent fusion genes.

This translocation did not remove the MLL gene. The breakpoint region between *PICALM* and *AF10* is represented in Figure 2. No anomaly was detected using next-generation sequencing of a restricted panel of frequently mutated genes in myeloid malignancies and, more specifically, epigenetic regulatory genes (*DNMT3A, EZH2, IDH1, IDH2*). Histology analysis following canthus tumefaction biopsy found undifferentiated blast cells expressing CD34, CD45, TdT, Bcl2, CD99, and C117 antigens (Figure 3). Consistent with detecting the clonal rearrangement of the T-cell receptor (TCR) gene, the diagnosis of early T-cell precursor lymphoblastic leukemia or granulocytic sarcoma was suspected. Brain MRI showed a unilateral and well-limited mass derived from the left lacrimal gland, (Figure 4) measuring 31 × 17 mm. FDG positron emission tomography (PET) revealed over and under diaphragmatic multiple adenopathy and numerous tissue involvements (Figure 5).

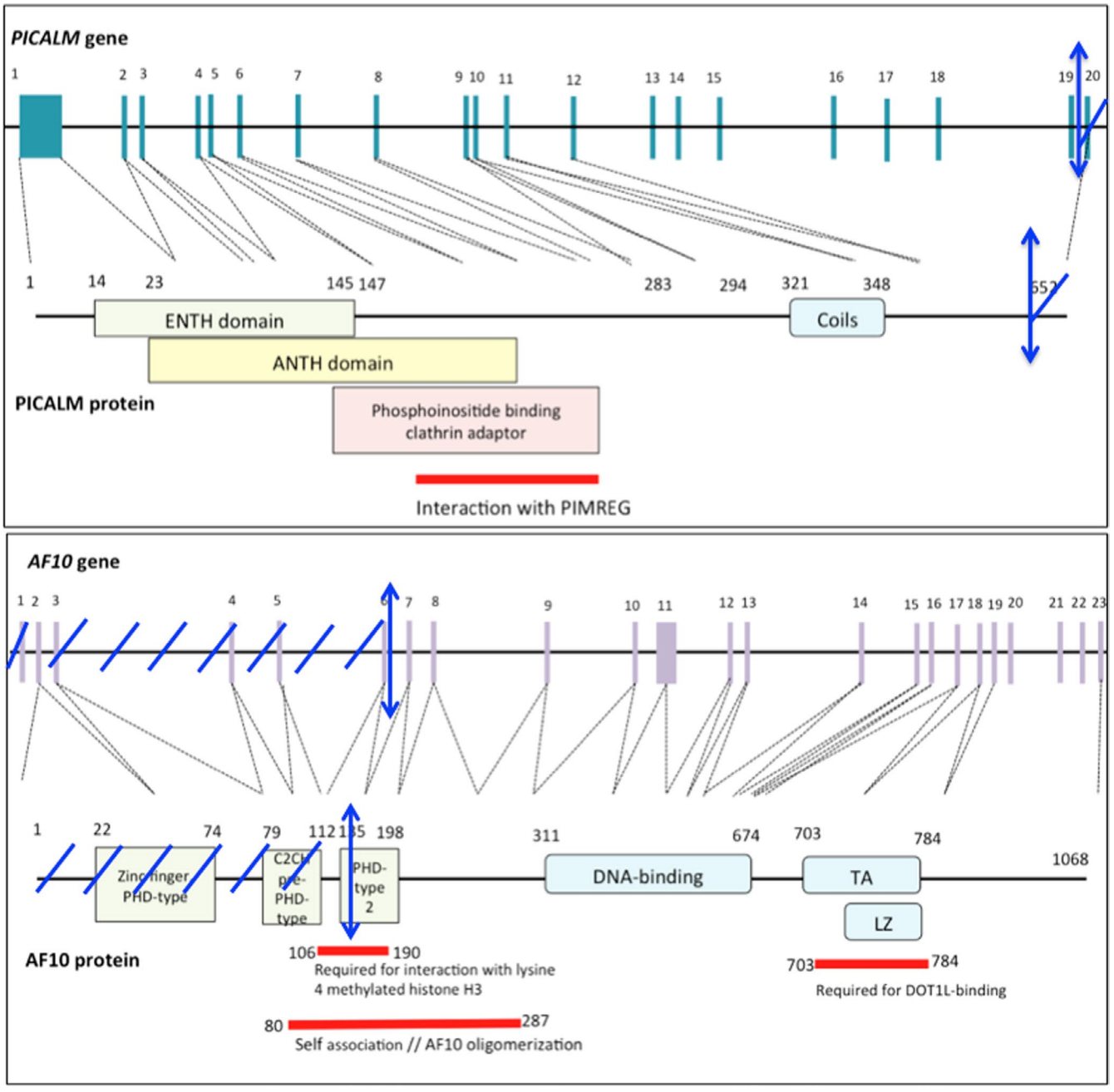

FIGURE 2 Representation of the *PICALM* gene with exons corresponding to PICALM functional domains. ENTH domain: epsin N-terminal homology domain. ANTH domain: AP180 N-terminal homology domain Representation of the *AF10* gene with exons corresponding to AF10 functional domains. The blue arrows represent translocation sites. PHD: plant homeodomain. TA: transactivation domain. LZ: octapeptide motif leucine-zipper. Reprinted from Uniprot.com and Ensembl.com.

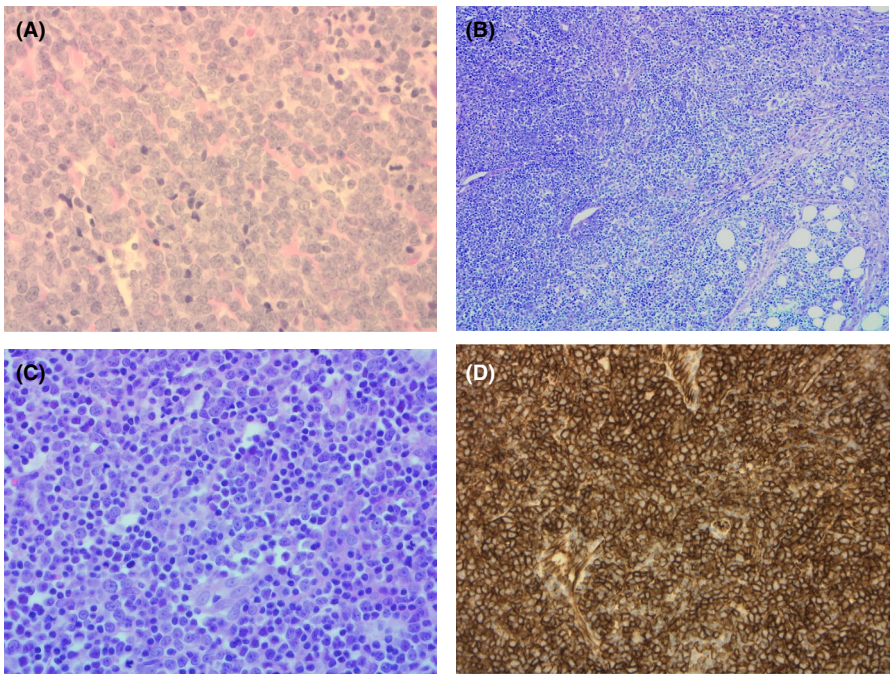

FIGURE 3 (A) Biopsy of the left lacrimal gland. Hematoxylin and eosin (H&E) ×40: Leukemic cells show no morphological features of myeloid or lymphoid differentiation and have several eccentrically placed nucleoli. (B) Lymph node H&E × 10 and (C) lymph node H&E × 40 biopsies show the same morphological aspect as in A. (D) Lymph node: immunohistochemical staining shows strong CD34 expression

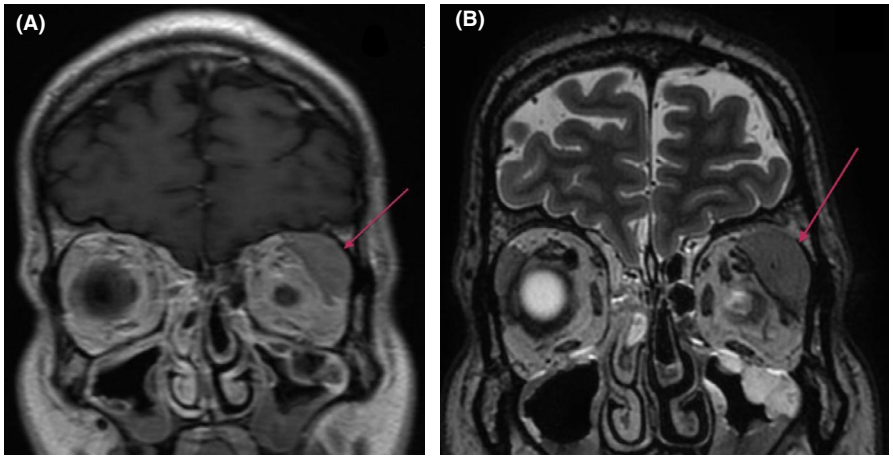

FIGURE 4 Brain MRI. (A) The left lacrimal gland shows hyperintensity on T1-weighted coronal imaging. (B) The left lacrimal gland shows isointensity on T2-weighted coronal imaging. (C) The left lacrimal gland shows high restriction on diffusion-weighted and diffusion-calculated (ADC) imaging

Because of diagnostic difficulties and uncommon clinical presentation, a second biopsy was performed. The histology of a lymph node concluded T/myeloid mixed-phenotype acute leukemia (T/M MPAL) with extranodal damage because of the weak positivity of CD4 and high-intensity clonal rearrangement of the T-cell receptor gene. The karyotype of this lymph node was the same as that of the bone marrow.

An AML-like chemotherapy regimen (3 + 7 with cytarabine and daunorubicin) with prophylactic intrathecal injections was started because of MPO positivity and myeloid aspect at cytology. The patient achieved complete molecular remission with negative minimal residual disease using *PICALM/AF10* monitoring by dedicated allele-specific quantitative PCR of bone marrow specimens (Figure 6) and a PET complete metabolic response at D30. The treatment was then completed with one cycle of consolidation chemotherapy comprising high-dose cytarabine (3 g/m^2 D1-D6) and matched unrelated donor hematopoietic stem cell transplantation, with myeloablative conditioning (busulfan 3,2 mg/kg/d D-7 to D-4 and cyclophosphamide 60 mg/kg/d D-3 to D-2). The patient is still in complete molecular remission, with a follow-up of 1 year after transplant.

3 | DISCUSSION

The diagnosis of mixed-phenotype acute leukemia (MPAL) is relatively difficult to establish. The WHO 2016 classification[4] defines acute leukemia of ambiguous lineage as leukemia showing no clear differentiation along a single lineage. This group includes acute undifferentiated leukemia and MPAL (or biphenotypic leukemia). MPAL is a rare and high-risk subtype of leukemia, accounting for less than 1%

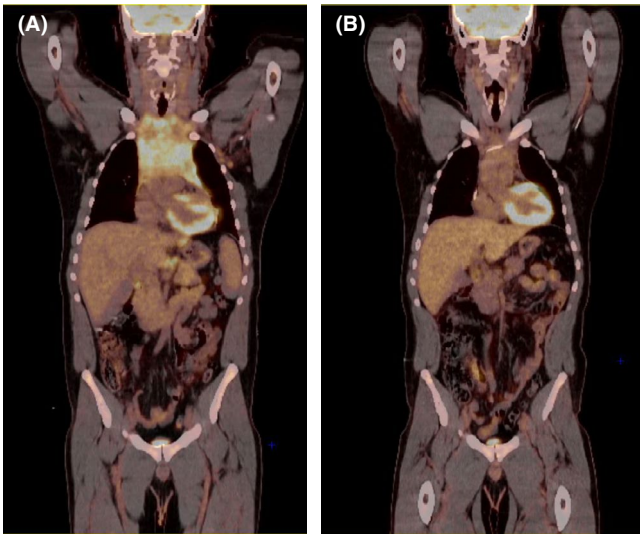

FIGURE 5 18F-Fluorodeoxyglucose positron emission tomography (PET) at baseline (A) and after induction chemotherapy (B) with a complete response

t(9;22)(q34;q11.2) *BCR-ABL1* and MPAL with t(v;11q23) *MLL*-rearranged (also known as *KMT2A*).[4] In our case, MLL was not rearranged. Kern *et al*[5] analyzed the specimens of 18 MPAL cases (T/M and B/M) by next-generation sequencing. Most of the genetic mutations identified affected predominantly epigenetic genes (*DNMT3A*, *TET2*, *IDH1/2*, *ASLX1*) or transcription factors (*TP53*, *RUNX1*, *ETV6*). *DNMT3A* was the most frequently mutated gene (55,6%; 8/16 MPAL cases), which encodes a DNA methyltransferase. It is mutated early in AML development and considered a founder mutation[6] associated with anthracycline resistance. In a pediatric population, Alexander et al[7] demonstrated by exome, transcriptome, or whole-exome sequencing that 100% of T/M MPAL presented alterations in genes encoding transcriptional regulators and 88% presented alterations in signaling pathways. They found similar genetic profiles between T/M MPAL and early T-cell precursor acute lymphoblastic leukemia (ETP-ALL),[7] particularly mutations in RAS and the Jak/STAT pathway.

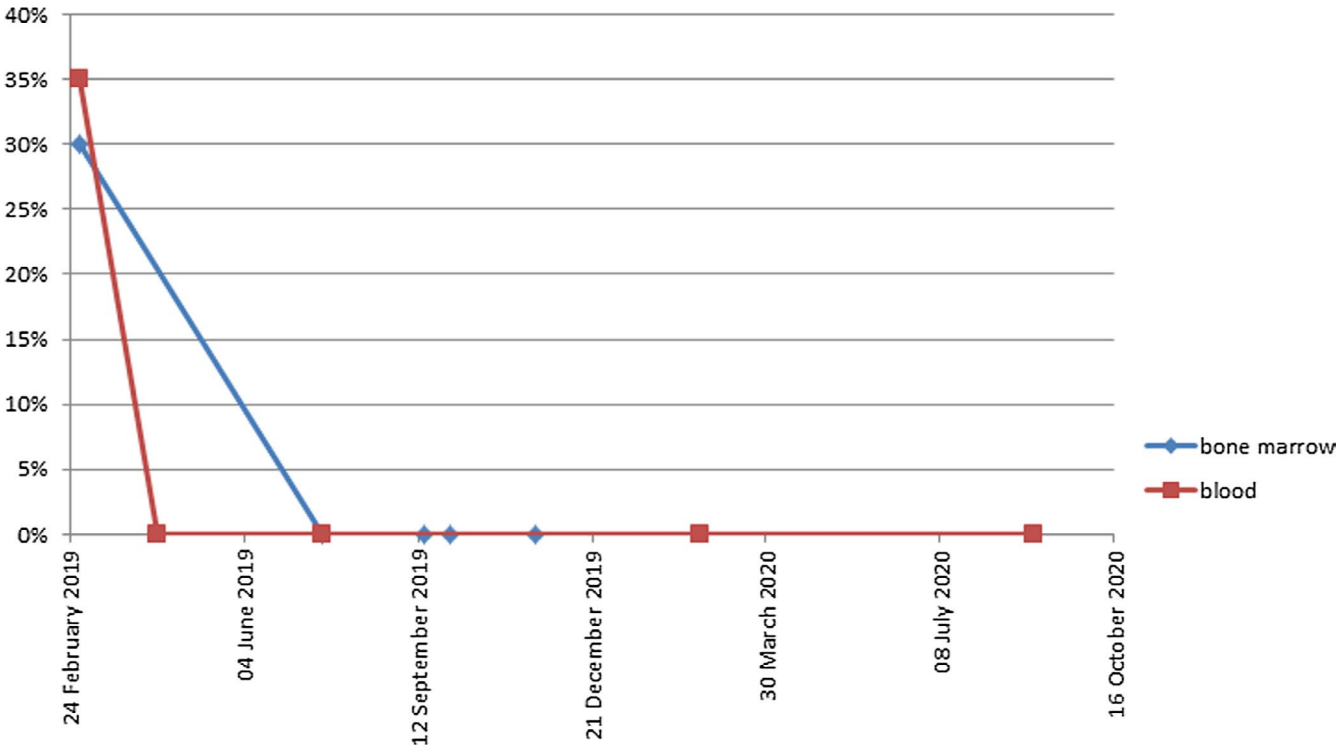

FIGURE 6 Dynamic detection of the PICALM-AF10 transcript by dedicated allele-specific quantitative PCR (qPCR) in the bone marrow and blood of the patient

of all leukemias.[1] The diagnosis requires expression of both lymphoid and myeloid differentiation antigens in the same leukemic cell by flow cytometry.[1] The long-term survival of MPAL is 47%–75% and 20%–40% for children and adults, respectively.

The genetic basis of mixed-phenotype acute leukemia remains unclarified, except for two distinct subsets: MPAL with

The translocation (10;11)(p13;q14) *PICALM/AF10* was first identified by Berger in 1989 in T-cell acute lymphoblastic leukemia. The identification of the same translocation in the U937 cell line originated from a diffuse histiocytic lymphoma[8] enabled Dreyling *et al* to determine the implicated genes: *AF10* at 10p13 and a new gene they named *CALM* (clathrin assembly lymphoid and myeloid

leukemia) at 11q14.[9] Several breakpoints exist: three on the *CALM* gene and four on the *AF10* gene.[10] Located at 11q14, the *CALM* gene has 24 transcripts by alternative splicing. It encodes 11 potential isoforms of a 652-amino acid ubiquitous protein called PICALM (phosphatidylinositol-binding clathrin assembly protein, or CALM). It is a clathrin adaptor protein that binds to the lipids in the plasma membrane and clathrin[11] and plays an important role in clathrin-mediated endocytosis. The *AF10* gene (*MLLT10*) at 10p13 encodes a putative transcription factor that binds DNA through an AT hook motif[12] and contains a nuclear localization signal. The translocation leads to the fusion of the protein PICALM with AF10 (MLLT10).

Several isoforms of *PICALM/AF10* have been detected, and all were associated with leukemogenesis.[10,13] Indeed, the translocation has been reported in hematologic diseases of distinct and various lineages, such as T- or B-cell acute lymphoblastic leukemia (T-ALL or B-ALL),[14] acute myeloblastic leukemia (AML),[15-17] MPAL[10,18,19] and lymphomas.[9] In Kumon *et al*'s study,[10] three of five patients with t(10;11) leukemia were young individuals, with a frequent mediastinal mass but no initial central nervous system involvement. Four of them did not respond to treatment (the treatment details were not reported). Interestingly, the immunophenotypes showed coexpression of T-cell and myeloid antigens in 80% of the cases.[13]

The detection of *PICALM/AF10* in multilineage hemopathologies favors stem cell or precursor damage. The transduction of mutant *CALM-AF10* cDNA in C57BL/6 mice bone marrow cells led to increased expression of lineage-uncommitted progenitors *in vitro*.[20] These cells did not express myeloid-specific or lymphoid-specific markers and displayed high levels of c-kit. Moreover, the translocation was often described as a simple karyotype, suggesting a driving role in the oncogenic process.[9]

At the molecular level, *PICALM/AF10* induces the overexpression of *HOXA* cluster genes (particularly *Hoxa5*) through aberrant methylation of Lys79 of Histone 3 via DOT1L recruitment.[20] *Hoxa5* overexpression is critical but not essential for *CALM-AF10*-mediated leukemogenesis.[21] In the Caudell *et al* study,[21] *CALM-AF10* transgenic mice showed *Hoxa5* overexpression, although they did not develop acute leukemia. The penetrance was incomplete, suggesting the need for additional events to trigger leukemic transformation. Other studies demonstrated the upregulation of *BMI1* in MPAL with t(10;11).[9,22] Bmi1 is a member of the polycomb repressive complex 1 (*PRC1*) family implicated in epigenetic control. This protein is constantly overexpressed in *PICALM/AF10* leukemia. The genetic *BMI1* depletion prevents and stops the *CALM-AF10*-mediated transformation of hematopoietic stem cells.[23]

Despite these molecular characterizations, no consensus exists regarding appropriate treatment for patients with T/M MPAL. The dilemma persists between the choice of AML and ALL-directed regimens,[7,24] although ALL therapy in the first line is currently often proposed.[3] The current hematopoiesis template does not explain the original cell of MPAL. The hypothesis of a myeloid template for hematopoiesis has been proposed in 2008 and 2009.[25,26] It postulates that one hematopoietic stem cell initially differentiates into erythroblastic/myeloid precursors and lymphoid/myeloid precursors. However, several authors[27] have demonstrated that the earliest thymic progenitors (ETP) have lymphoid and myeloid potential, arguing in favor of the persistence of transcriptional promiscuity even after lineage commitment. In our report, the AML-like regimen induced complete remission of T/M MPAL with t(10;11)(p13;q14) *PICALM/AF10*, supporting the case for a myeloid template and the hypothesis of a multipotent precursor.

4 | CONCLUSION

MPAL is a very heterogeneous group, comprising T/M, B/T MPAL and subtypes within each group. The T/M MPAL physiopathology remains unclear, and further research is ongoing to unravel new effective treatments. Several therapeutic targets have been disclosed, such as H3K79 methylation alterations, *BMI1,* and *HOXA*. Several hypotheses may be required to lead to an apparent identical result. T/M MPAL could originate from a multipotent precursor in some cases or ETP with myeloid potential in other cases. The detection of *PICALM/AF10* in multilineage hematological malignancies favors precursor damage. Our report supports the myeloid template and hypothesis of a multipotent precursor for T/M MPAL with t(10;11)(p13;q14) *PICALM/AF10*.

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CONFLICT OF INTEREST
None declared.

AUTHOR CONTRIBUTIONS
DK and VC: wrote the manuscript. FJ: reviewed the manuscript. All authors were involved in the care of the patient. All authors read and approved the final manuscript.

ETHICAL STATEMENT
Patient's written informed consent to publication was obtained.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available on request from the corresponding author, VC. The data are not publicly available due to their containing information that could compromise the privacy of the patient.

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