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A paradigm shift: lineage switch from T-ALL to B/myeloid MPAL

TO THE EDITOR: Lineage switch in acute leukemia is de-fined as a complete immunophenotypic change either at relapse or during therapy. Lineage switch from acute lym-phoblastic leukemia (ALL) to acute myeloid leukemia (AML) is observed in most cases; the reverse is uncommon [1, 2]. The exact mechanism underlying lineage switch is unclear [3]. Lineage switch from T-ALL to mixed-phenotype acute leukemia of B/myeloid lineage (B/myeloid MPAL) is excep-tionally rare. We report a case of T-ALL at initial pre-sentation, which showed lineage switch to B/myeloid MPAL at relapse. We believe that this is the first such case to be reported.

A 15-year-old boy was initially presented to our hospital in 2007 with fever and breathing difficulty. Clinical exami-
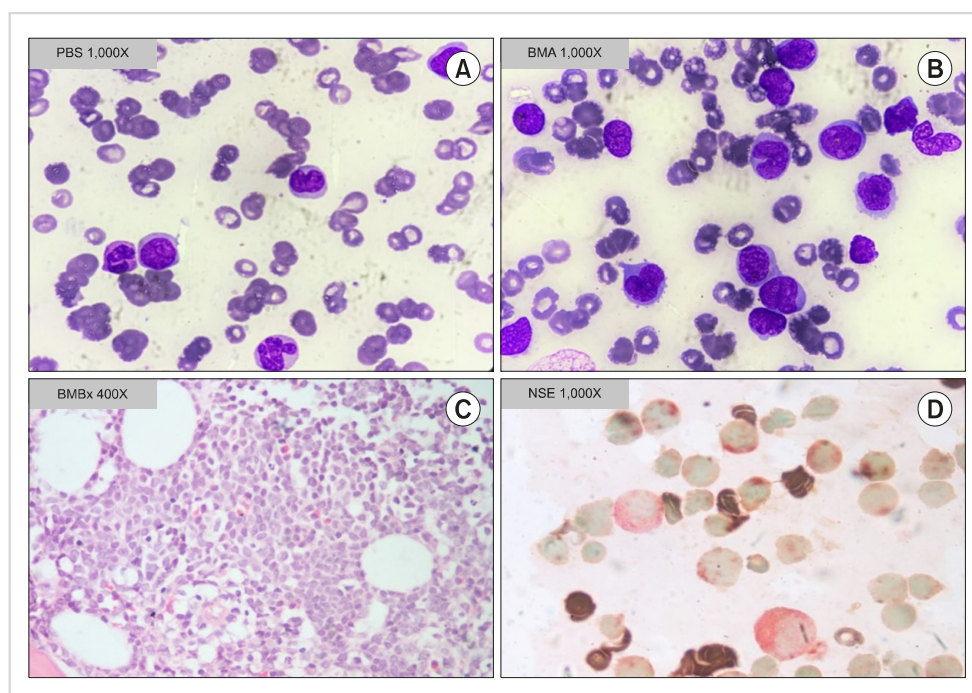

Fig. 1. Peripheral blood and bone marrow morphology. Peripheral blood smear showing blasts and monocytes **(A)**. Bone marrow aspirate showing blasts and monocytic population **(B)**. Bone marrow biopsy showing marrow spaces replaced by blasts **(C)**. NSE positivity in blasts **(D)**.

nation showed bilateral cervical lymphadenopathy and hepatosplenomegaly. Contrast-enhanced computed tomography (CECT) showed mediastinal lymphadenopathy with bilateral pleural effusion. The hemogram showed a white blood cell (WBC) count of 19.5×10^9/L, a hemoglobin level of 11.0 g/dL, and a platelet count of 171×10^9/L. No blasts were observed in the peripheral blood film. The bone marrow aspirate and biopsy showed >80% blasts. These blasts were negative for myeloperoxidase (MPO), Sudan Black B (SBB), non-specific esterase (NSE), and periodic acid-Schiff (PAS) stain. Flow cytometric immunophenotyping of the bone marrow was performed at an external diagnostic center, which showed an immunophenotype consistent with precursor T-ALL. The blasts were positive for CD34, cytoplasmic CD3, CD5, CD7, and CD45 and negative for CD19, CD20, CD10, CD117, CD22, CD33, and MPO. Karyotype analysis was unsuccessful as no metaphase chromosomes were detected in the culture. Genetic studies were negative for 11q23 (*MLL*) gene rearrangement (11q23 probe; Cancer Genetics Inc., Rutherford, NJ, USA). The central nervous system was not involved. The patient was treated with an appropriate chemotherapy protocol (hyper-CVAD regimen). He achieved remission after 8 cycles of chemotherapy with complete blood count recovery. Subsequently, he was lost to follow-up at our hospital and reportedly continued treatment elsewhere. He was doing well post-treatment until mid-2020.

The patient was again admitted to our hospital in mid-2020 (13 years after the initial presentation in 2007)

with complaints of fever and inguinal lymphadenopathy. No hepatosplenomegaly was observed on clinical examination. He had a WBC count of 16.0×10^9/L with 18% blasts and monocytosis (Fig. 1A). Hemoglobin and platelet counts were 5.3 g/dL and 60×10^9/L, respectively. The bone marrow aspirate showed two distinct abnormal populations on morphological examination; the first population consisted of medium-sized, round agranular blasts (65% of all nucleated cells), and the second population consisted of monoblasts, promonocytes, and abnormal monocytes (30% of all nucleated cells) (Fig. 1B, C). Flow cytometric immunophenotyping performed on the bone marrow sample showed two distinct abnormal cell populations (Fig. 2). The first population (45% of all viable singlets) showed negative to moderate CD45 expression and low side scatter (progenitor population). The cells expressed CD19, CD10, cCD79a, CD34, TdT, HLA-DR, CD13, and CD64 and were negative for c-MPO, CD117, CD33, cytoCD3, CD7, CD11c, CD2, and CD5. The second population was detected in the monocyte region and included 20% of all viable singlets. The monocytic population expressed CD34, CD64, and CD11c (dim). On cytochemistry, these blasts were positive for NSE (Fig. 1D) and negative for MPO and PAS stain. A diagnosis of B/myeloid MPAL was made. Cytogenetic analysis by conventional karyotyping showed no structural chromosomal anomalies. Targeted next-generation sequencing (NGS) showed mutations in *ASXL1* (c.1926_1927insG), *ETV6* (c.313_314insTGGGCCCT), and *RUNX1* (c.592G>A, IKZF1c.476A>C) with mutant allele percentages of 32.8%, 41.5%, and 45.2%, respectively.
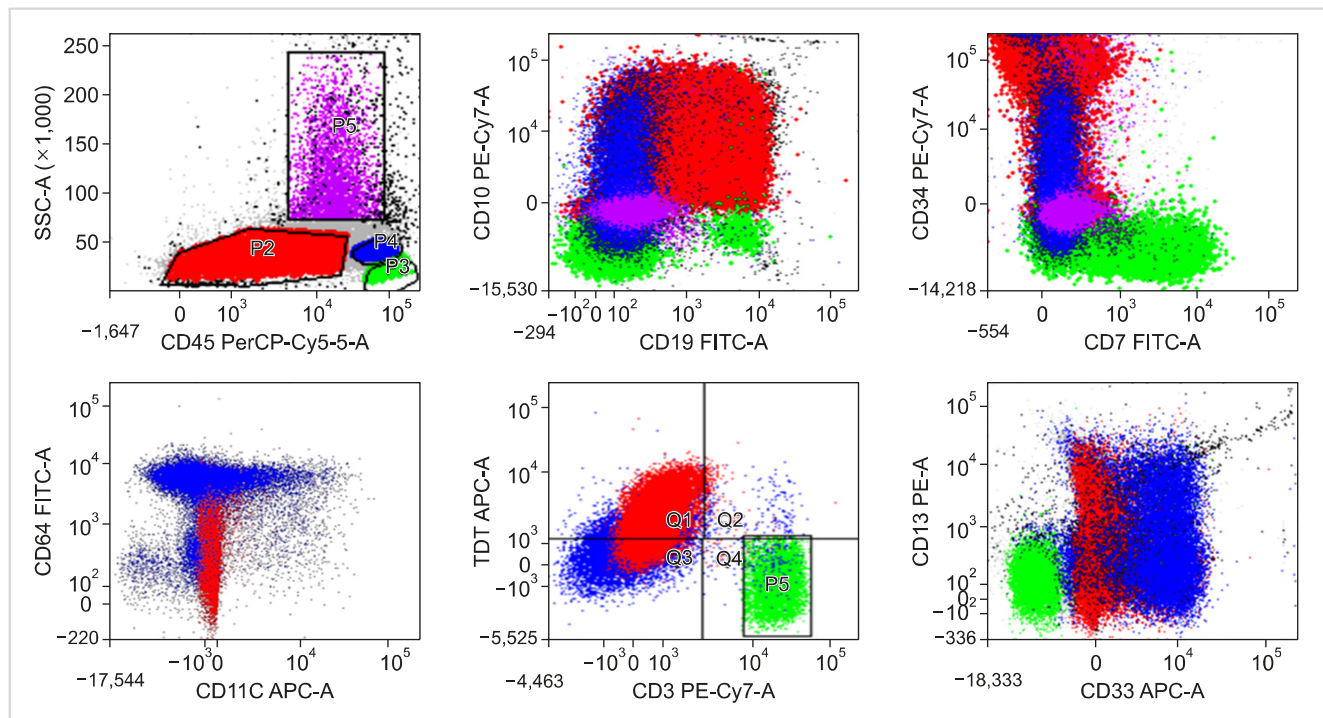

Fig. 2. Flow cytometric immunophenotyping plots. Flow cytometric immunophenotyping identified two abnormal populations, i.e., B-lymphoblasts (red) and monoblasts/promonocytes (blue), indicating B/myeloid MPAL.

The patient was treated using an augmented Berlin-Frankfurt-Münster (BFM) chemotherapy protocol. On day 29 of treatment (post-induction therapy), the bone marrow showed complete remission with incomplete platelet recovery (platelet count, 25×10^9/L).

MPAL accounts for 2–4% of all acute leukemia cases, and prognosis is poor compared with that for ALL or AML [4]. MPAL is genetically extremely heterogeneous. According to the World Health Organization (WHO), a diagnosis of MPAL requires the expression of lineage-specific markers in at least two lineages [4]. Lineage switch occurs in 6–9% of acute leukemia cases during relapse [3]. Switching from ALL to AML is associated with *MLL* gene rearrangement in most cases, especially in cases of pediatric leukemia [5, 6]. These cases are associated with poor survival. Lineage switch may occur due to a highly plastic original clone or the emergence of a new clone; however, the exact mechanism remains unclear [3]. One of the several hypotheses suggests that the original committed neoplastic clone is highly plastic, and phenotypic changes may be observed with or without changes in the genotype [3]. The possible role of the microenvironment in the switch is also suggested [3]. In clonal selection theory, the emergence of resistant subclones with different lineages at relapse has been hypothesized [7]. In B-ALL to myeloid conversion or vice versa, the role of a common B-myeloid progenitor is suggested. Myeloid switch from B-ALL can occur either by trans-differentiation, de-differentiation, or re-differentiation [8]. Lineage switch in acute leukemia is uncommon, partly because repeat immunophenotyping is not always performed at relapse. Most of the information found in the published literature describes a switch from B-ALL to AML or the reverse. Some studies reporting a switch from T-ALL to AML have been published [9-11]. However, lineage switch from T-ALL to B/myeloid MPAL appears to be extremely rare. To the best of our knowledge, this has not been published previously.

Relapse in childhood acute leukemia usually occurs within 2 years and is thought to arise from the same clone as that at the initial presentation. Late relapse may indicate a completely new clone, which is characteristic of secondary leukemia and associated with a prior history of cytotoxic therapy [12]. The presence of therapy-related myeloid neoplasms (t-MNs) is a late effect of chemotherapy (alkylating agents/topoisomerase II inhibitors) and/or radiation after the treatment of a primary disease (post-transplant lymphoproliferative disorders following solid organ transplantation) [13]. t-MNs are chemoresistant and have an aggressive course with a survival time of several months [13]. t-MNs associated with treatment using alkylating agents almost always have a dysplastic component in the marrow with cytogenetic anomalies such as del5q, del7q, or monosomy 7 on karyotyping [14]. Secondary ALL has been suggested to be constitutional and not related to any prior therapy [15]. In this case, targeted NGS analysis did not show any findings suggestive of germline mutations or constitutional abnormalities. The patient did not have any persistent symptoms attributable to cytopenias of myelodysplastic syndrome (MDS) prior to the second presentation. Karyotyping analysis at relapse did not reveal any MDS-defining cytogenetic abnormality, which is common in therapy-related myeloid neoplasms following the use of alkylating agents. Therefore, constitutional genetic abnormalities, MDS, and t-MNs were less likely in this case. The emergence of a resistant subclone at relapse or a highly plastic original clone may be responsible for the lineage switch in this case.

Cases of leukemia with lineage switch may have distinct biological characteristics and clinical features and may be associated with a poor prognosis and response to therapy [1]. Changes in the blast morphology and immunophenotype suggested lineage switch in this case. The recognition of this event may help to guide appropriate investigation and planning for therapy. Overall, the findings demonstrated the importance of repeat immunophenotyping even with acute leukemia relapse to identify relevant cases for proper management.

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Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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Utility of platelet cross-matching in a case of neonatal alloimmune thrombocytopenia associated with a de novo MECOM variant

TO THE EDITOR: Neonatal alloimmune thrombocytopenia (NAIT) is the most important cause of thrombocytopenia in term neonates. It was estimated to occur at a frequency of 1 in 1,000–2,000 live births [1]. Platelet destruction is caused by a maternal antibody directed against a fetal human platelet antigen (HPA) inherited from the father and lacking in the platelets of the mother. In the Caucasian population, 80% alloimmunization occurs predominantly due to antibodies to the HPA-1a antigen [1]. Although less common,

the HPA-15 antigen system is also implicated in serologically confirmed NAIT cases [2]. The HPA-15a15b system is located on the glycosyl-phosphatidylinosotol-anchored protein, CD109, and is defined by the single amino acid substitution of Ser703Tyr (Ser682Tyr of mature CD109 protein) [3]. The diagnosis of NAIT can be established by documenting the presence of a platelet-specific antigen incompatibility between mother and infant (or mother and father) and the presence of maternal antiplatelet allo-antibodies directed against the incompatible HPA antigen [4]. The monoclonal antibody-specific immobilization of platelet antigen (MAIPA) assay is considered as the 'gold-standard' test to detect maternal anti-HPA antibodies [4]. However, it is not available for routine use in India. Platelet cross-match, which is commonly used to provide platelet transfusion support in platelet refractoriness, may be considered as an alternative strategy to manage emergency cases of NAIT in resource-limited facilities where a platelet-donor registry is still unavailable [5].

In 2015, heterozygous mutations in the MECOM (MDS1 and EVI1 complex locus) were first identified as a cause of amegakaryocytic thrombocytopenia in neonates [6]. There are many novel mutations in the MECOM gene that have been identified recently, and a new name, 'MECOM-associated syndrome,' has been proposed for this disease [7]. Herein, we report a rare case of NAIT associated with HPA-15b incompatibility in a baby who was managed initially with the transfusion of cross-matched platelets, but due to persistent transfusion-dependent thrombocytopenia and inadequate response to appropriate therapy such as IVIG, whole exome sequencing was performed, which re-

Table 1. Platelet cross-match, platelet antibody screening and HPA genotyping results.

Tests	Results
Platelet antibody screening with baby's serum	Positive
Platelet antibody screening with mother's serum	Positive
Mother's serum cross-matched with baby's platelets	Incompatible
Mother's serum cross-matched with husband's (father's) platelets	Incompatible
Baby's serum cross-matched with father's platelets	Incompatible
Incompatibility % during platelet cross-matching: from day 1 to day 20	50% (8/16)
Incompatibility % during platelet cross-matching: from day 21 to day 40	30% (6/20)
Incompatibility % during platelet cross-matching: from day 41 to day 61	18.2% (4/22)
HPA genotyping of baby and parents to detect HPA incompatibility in the baby	HPA-15b incompatibility detected in the baby

Abbreviation: HPA, human platelet antigen.