De novo primary central nervous system pure erythroid leukemia/sarcoma with t(1;16)(p31;q24) *NFIA/CBFA2T3* translocation

We report an unusual case of *de novo* pure erythroid leukemia/sarcoma (PEL/PES) presenting solely as primary central nervous system (CNS) disease. This is the second PEL case with confirmed t(1;16)(p31;q24) *nuclear factor I A (NFIA)/core binding factor A2T3 (CBFA2T3)* translocation.[1] We performed comprehensive molecular profiling from tumor cells obtained from cerebrospinal fluid (CSF) which identified a *NFIA/CBFA2T3* fusion and somatic variation in *EPOR, Janus kinase 2 (JAK2),* and *ARID1A*.

This 2-year-old female presented with vomiting. A head computerized tomography (CT) scan and magnetic resonance imaging (MRI) showed a well-circumscribed hyperdense mass (3.7x2.5x1.5 cm) within the posterior third ventricle/tectum with a pineal epicenter (Figure 1A). There was diffuse leptomeningeal dissemination. Given the negative prior history, laboratory studies (normal complete blood count [CBC] and clinical chemistry) and extra-cranial imaging findings, a primary CNS tumor was presumed.

Endoscopic biopsy showed a poorly differentiated malignant neoplasm with mitoses and necrosis (Figure 1B). Except for patchy CD117 and EMA by immunohistochemistry (IHC), the tumor was negative with an extensive antibody panel but highly proliferative (Ki-67 >90%). Further workup showed tumor cells were diffusely CD43-positive with focal CD45 and CD33 expression, but negative for other hematopoietic markers (Figure 1A). Myeloid sarcoma (MS) was favored. A bone marrow evaluation was negative for hematopoietic neoplasia.

Lumbar puncture showed that CSF was hypercellular, with numerous large, pleomorphic erythroblasts confirmed by flow cytometric immunophenotyping (Figure 2A-B). These cells showed scant to moderate basophilic to polychromatic cytoplasm, the latter likely from hemoglobin accumulation. Cytoplasmic blebbing was frequent. Multinucleation, nuclear budding and mitotic figures were seen. Flow cytometry detected an aberrant cell population (90%) expressing CD36 (bright), CD71, heterogeneous CD117, heterogeneous glycophorin A; the population was negative for CD14, CD15, CD34, CD38, CD45, and CD64. CSF findings prompted additional IHC staining of the brain biopsy. Tumor cells were positive for E-cadherin, CD71, and hemoglobin (variable) (Figure 1B). A definitive diagnosis of PEL/PES was rendered just over two weeks later.

Cytogenetic studies on CSF detected a hyperdiploid clonal cell population (Figure 2C): 54,XX,+X,t(1;16)(p31;q24),+6,+7,+8,+8,+10,+14,+19[12]/55,sl,+15[8]. While there was no overt leukemia in the bone marrow, the same abnormal clone was detected at low levels: left aspirate (53,XX,+X,t(1;16)(p31;q24),+6,+7,+8,+10,+14,+19[1]/46,XX[19]) and right aspirate(53,XX,+X,t(1;16)(p31;q24),+6,+7,+8,+10,+14,+19[1]/54,sl,+8[2]/54,sl,+17[cp2]/46,XX[15]).

RNA-sequencing of tumor cells identified an in-frame fusion between exon 4 of NFIA (NM_001134673.4) and exon 2 of *CBFA2T3* (NM_005187.6) which was confirmed by reverse-transcriptase PCR (RT-PCR) followed by Sanger sequencing (Figure 3A and *Online Supplementary Materials and Methods*). Notably, the *CBFA2T3* fusion breakpoint was identical to a previously published case report.[1] Using whole genome sequencing (WGS), we further delineated the breakpoints to within intron 4 of NFIA (GRCh37 chr1:61817174) and intron 1 of *CBFA2T3* (GRCh37 chr16:88975717). We also identified the reciprocal fusion (CBFA2T3/NFIA), also confirmed by Sanger sequencing. The estimated 835 amino

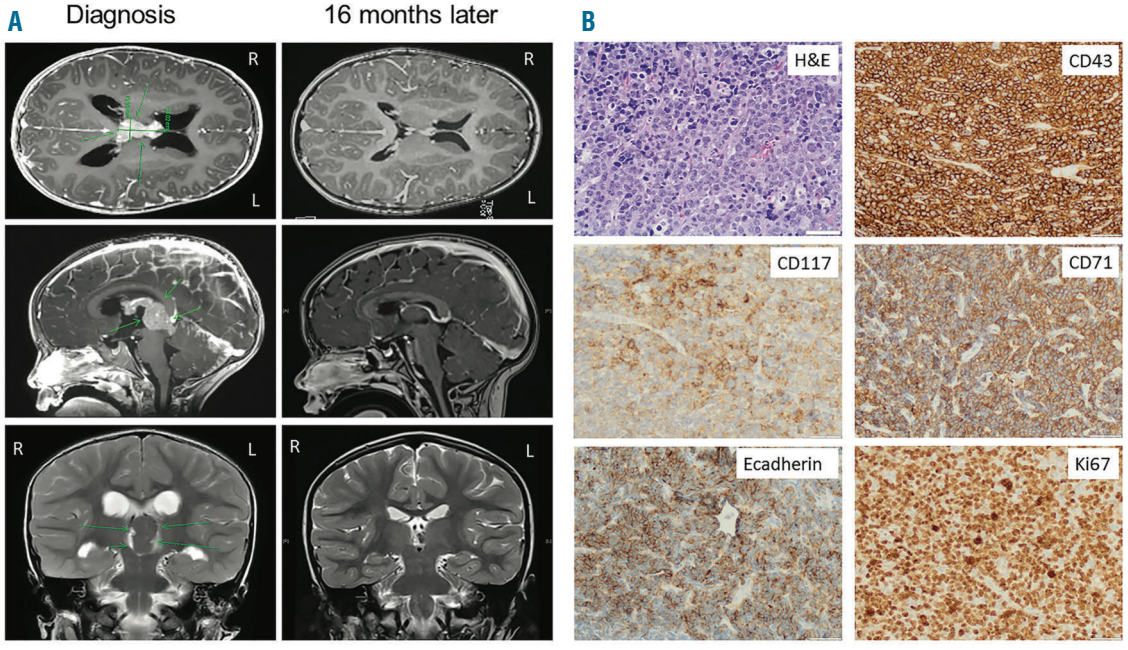

Figure 1. Radiology and pathology of brain mass. (A) Magnetic resonance imaging (MRI) images of the brain mass. Top: axial T1-weighted with contrast, middle: sagittal T1-weighted with contrast, bottom: coronal T2-weighted. (B) Pathology of the brain mass biopsy showed a poorly differentiated malignant neoplasm. The tumor cells had scant cytoplasm, highly pleomorphic nuclei with irregular nuclear contours, condensed to open chromatin and prominent nucleoli. There were increased mitoses with abnormal forms and increased apoptoses (400x, hematoxylin and eosin [H&E] stain and immunohistochemistry [IHC]). The tumor cells were positive for CD43, CD71, patchy CD117, and E-cadherin, with a high proliferative index (Ki-67 >90%).

acid chimeric protein is predicted to truncate within the C-terminal CCAAT box-binding transcription factor (CTF)/NF1 domain of NFIA (NP_001128145.1) while retaining the evolutionarily conserved nervy homology region (NHR) domains within *CBFA2T3* (NP_005178.4) (Figure 3B). *NFIA* is a transcription factor that promotes erythroid differentiation and represses granulocytic differentiation in human hematopoiesis by activating expression of several erythroid-specific genes.[2] Genetic variation and fusions of *NFIA* are reported in different cancers.[3] *CBFA2T3* is a member of the myeloid translocation gene family of transcriptional co-repressors.[4] *CBFA2T3* does not bind DNA directly. Instead, it is recruited to target genes by interacting with other transcriptional factors. Recombination of *CBFA2T3* with *runt-related transcription factor 1 (RUNX1)* and *GLIS family zinc finger 2 (GLIS2)* has been demonstrated in acute myeloid leukemia (AML).[4,5] The t(1;16)(p31;q24) balanced translocation was previously reported in three children with PEL.[1,6] Thus, detection of t(1;16)(p31;q24) encoding *NFIA/CBFA2T3* in our patient lends support that this is likely a PEL/PES-specific recurrent rearrangement.

Additionally, we performed WGS from comparator normal blood and tumor cells derived from CSF to assess for genomic variation and copy number alterations (*Online Supplementary Materials and Methods*). The copy number profile was consistent with the reported karyotype, revealing gains of chromosome X, 6, 7, 8, 10, 14, and 19 (*Online Supplementary Figure S1*). A full list of high-quality somatic variants can be found in the *Online Supplementary Table S1*. We identified somatic variants in *EPOR* (NM_000121.3:c.1316G>A:p.Trp439Ter) and *JAK2* (NM_004972.3:c.2651T>C:p.Leu884Pro) (Figure 3C). Two somatic alterations were detected in *ARID1A*, a nonsense variant (NM_006015.4:c.2231 C>G:p.Ser744Ter) and a ~24.5kb deletion (NG_029965.1 (NM_006015.4):c.1920+9172_2878+15del) including exons 5-9 (Figure 3C-D). The *EPOR* gene encodes the erythropoietin receptor essential for normal erythroid development.[7] Germline inheritance of EPOR p.Trp439Ter was previously reported in a large kindred with primary familial congenital polycythemia.[7] This variant encodes a truncated protein hypersensitive to erythropoietin, resulting in constitutive JAK-STAT activation and erythrocytosis. Similar deletions with loss of the C-terminus have been described in Philadelphia-like B-lymphoblastic leukemia (St. Jude PeCan Database). *JAK2* is a non-receptor tyrosine kinase.[8] *JAK2* p.Val617Phe mutation within the pseudo-kinase domain, is frequently detected in individuals with polycythemia vera and myeloproliferative disorders.[8] The novel *JAK2* p.Leu884Ser variant identified in our case is located within the kinase domain and has not been functionally characterized; however, alterations within the kinase domain have been reported in hematologic disorders. *ARID1A* encodes a subunit of the chromatin-remodeling SWI/SNF complex.[9] Mutation of *ARID1A* is detected in many tumor types, predominantly carcinomas, and might be a target for therapy.[10] The nonsense mutation in our patient is pathogenic and was detected previously in three patient tumors (breast duct carcinoma, pulmonary squamous cell carcinoma, and gastric adenocarcinoma).[10]

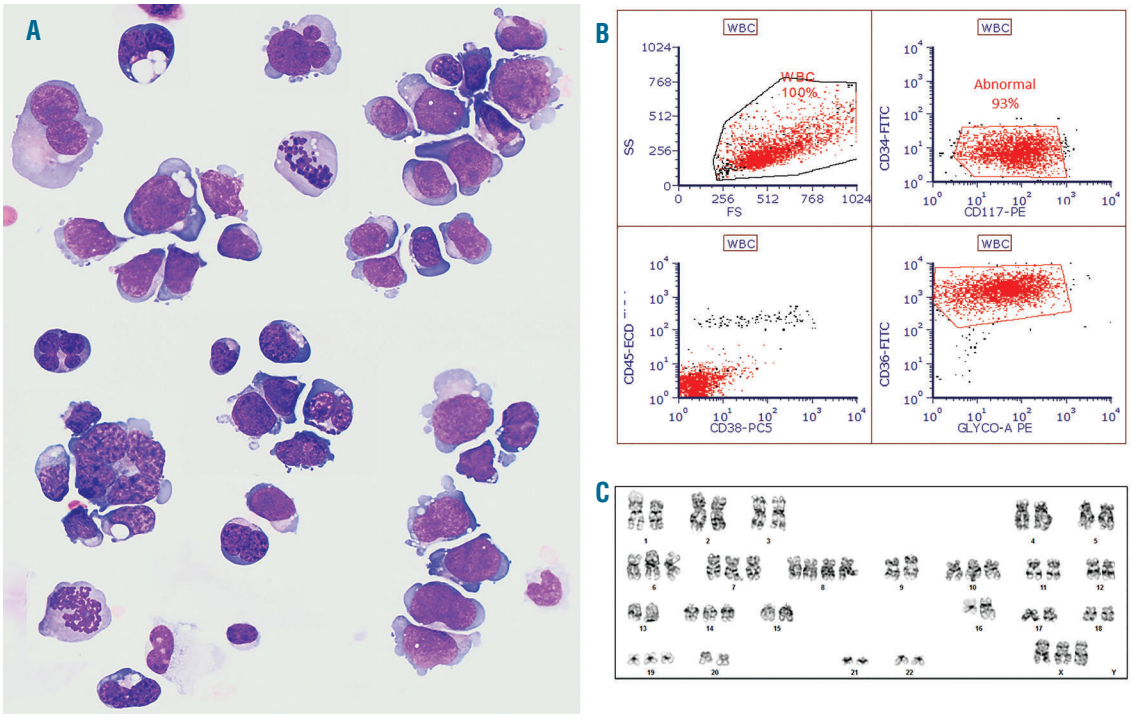

Figure 2. Tumor cells from from cerebrospinal fluid. (A) Cerebrospinal fluid (CSF) cytospin preparation (1000x, Diff-Quick stain) shows abnormal proerythroblasts and erythroblasts. The proerythroblasts are of medium to large size, display dispersed chromatin, some with prominent nucleoli. The erythroblasts are smaller with more condensed chromatin. Bi- or multi-nucleation and nuclear budding is seen. The erythroblasts have scant to moderate basophilic or polychromatic cytoplasm, the latter likely due to the accumulation of hemoglobin. Cytoplasmic vacuoles are seen in a subset of blasts. Cytoplasmic blebbing or pseudopods are frequent. Mitotic figures are seen. (B) Flow cytometry dot plot of CSF-specimen. Flow cytometric analysis detected an aberrant population (90% of total events) expressing CD36 (bright) CD71, heterogenous CD117, heterogeneous glycophorin A, negative for CD14, CD15, CD34, CD38, CD45, and CD64. (C) Karyotyping of CSF tumor cells. SS: side scatter; FS: foward scatter.

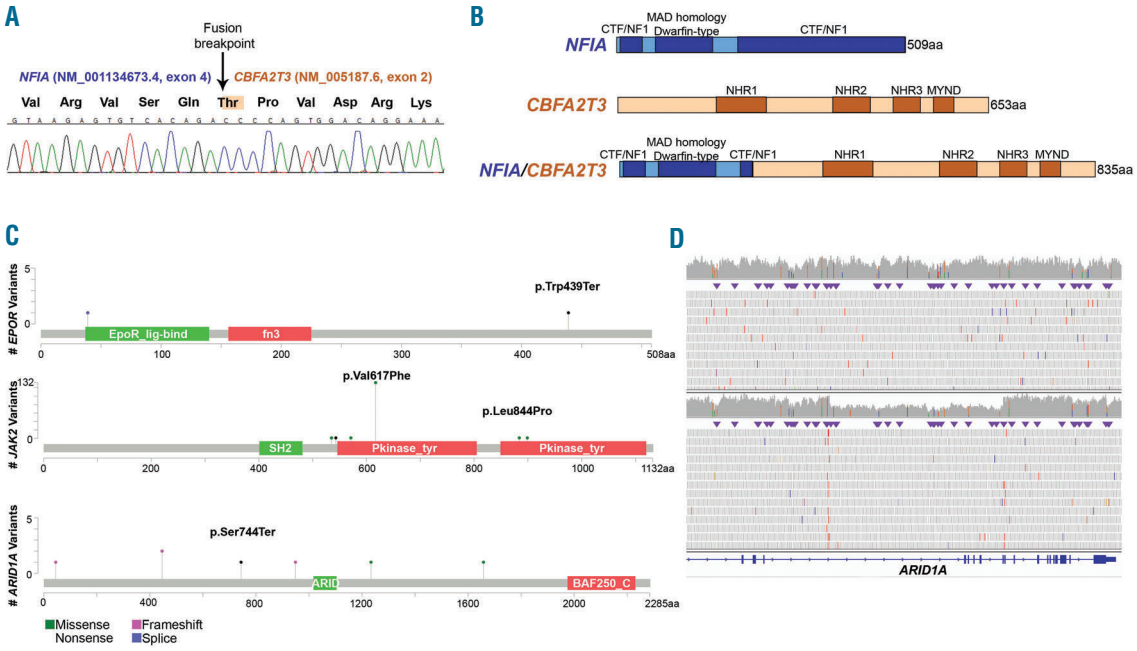

Figure 3. Molecular characterization of tumor. (A) Sanger sequencing chromatogram of the *NFIA/CBFA2T3* fusion depicting and in-frame fusion of exon 4 of *NFIA* (NM_001134673.4) to exon 2 of *CBFA2T3* (NM_005187.6). (B) Chimeric protein of *NFIA/CBFA2T3* fusion predicted to truncate within the C-terminal CCAAT box-binding transcription factor (CTF)/NF1 domain of *NFIA* and retains all nervy homology regions (NHR) and the Myeloid, Nervy, and DEAF-1 (MYND) domain of *CBFA2T3*. (C) Lollipop plots of *EPOR, JAK2,* and *ARID1A* describing the somatic variants identified in our case (denoted by the arrow) and those associated with myeloid disorders in cBioPortal. (D) Visualization of a 24 kb deletion within ARID1A. Top: Next generation sequencing of comparator normal blood. Bottom: Next generation sequencing of the tumor.

None of these findings have been reported in PEL.[10] Molecular mutation analyses have only been performed on small numbers of PEL due to its rarity.[11] Our case expands the mutational spectrum of PEL, as well as potential targets for therapy.

Initial induction chemotherapy was administrated according to AAML1031 with twice weekly intrathecal injection (IT) until clear plus two additional injections. Marked cytoreduction of intracranial primary and metastases was achieved. CSF turned negative in two weeks. The end of induction bone marrow was negative. She received induction II chemotherapy according to AAML1031-high risk arm and proceeded to matched sibling bone marrow transplant (BMT) six months later. A surveillance lumbar puncture revealed an isolated CSF relapse two months post-BMT. She received additional IT chemotherapy and radiation. She currently remains in complete remission (20 months after CSF relapse, 28 months after initial presentation).

The unique scenario of our case bestowed significant challenges in diagnosis. Considering the main tumor mass was in the brain, and tumor cells were morphologically inconspicuous in the bone marrow, it seems most appropriate to designate this neoplasm as a *de novo* primary CNS tumor. The completely normal CBC in this patient at presentation supports this contention as well, as it would be highly unusual to present with a relatively large brain mass and a normal CBC, had a bona fide leukemic process been initiated in the bone marrow. Primary de novo CNS myeloid sarcoma without prior or concurrent myeloid neoplasm is rare with less than 20 cases reported, predominantly in children.[12] Pineal/third ventricles are uncommon sites for myeloid sarcoma with only two cases reported,[12,13] a young child with *de novo* disease and an adult following prior AML. *De novo* pure erythroid sarcoma was only reported in five pediatric patients so far, with concurrent or metachronous leukemia in four cases (4 of 5), and tropism for orbit and/or skull (4 of 5).[14,15] CSF is key to diagnosis, and follow-up; especially for unusual tumors with diffuse leptomeningeal dissemination.

Lack of standard protocol for this rare disease posed challenges for the therapy. Despite the diagnosis of PES/PEL with a complex karyotype, commonly associated with dismal prognosis, our patient is in complete remission. This indicates PES/PEL is not necessarily terminal when treated optimally. A similar case of primary *de novo* CNS pure erythroid sarcoma was reviewed in consultation by one co-author (SK). The patient was a three-year-old female who presented with diffuse leptomeningeal dissemination, without a discrete mass. The diagnosis was made by CSF cytology. Her bone marrow was negative. She remains in remission, following chemotherapy, 10 years later. Our case provides an example for successful clinical management of similar patients in the future.

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