# Case Report

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# Childhood Therapy—Related Acute Myeloid Leukemia with t(16;21)(q24;q22)/*RUNX1-CBFA2T3* After a Primitive Neuroectodermal Tumor of the Chest Wall

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## Clinical Practice Points

- The t(16;21)(q24;q22) is a rare nonrandom chromosomal translocation, generating the *RUNX1-CBFA2T3* (runt-related transcription factor 1—core binding factor runt domain alpha subunit 2) fusion gene that is typically associated with therapy-related acute myeloid leukemia (t-AML) in adults.
- In the pediatric setting, it has been almost exclusively reported in cases of de novo AML; further, only 4 cases of childhood t-AML with t(16;21)(q24;q22)/*RUNX1-CBFA2T3* have been described to date.
- We report here the first case of t(16;21)(q24;q22)/*RUNX1-CBFA2T3* occurring in a 2-year-old with Askin tumor, a uncommon primitive neuroectodermal tumor of the chest wall belonging to the Ewing sarcoma family of tumors (EFT).
- Given the extreme rarity of pediatric t(16;21)(q24;q22)/*RUNX1-CBFA2T3* t-AML, we present a summary of the 4 previous cases, highlighting that all patients but one had an EFT member as primary tumor.
- Patients with EFT are usually treated with time-intense chemotherapy regimens, which, by combining topoisomerase II inhibitors and alkylators, are associated with a significant risk of developing therapy-related malignancies such as the rare t-AML subtype described here.
- Clinicians are encouraged to strictly monitor pediatric EFT patients after primary treatment because they represent a high-risk group for t(16;21)(q24;q22)/*RUNX1-CBFA2T3* t-AML.

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## Introduction

t(16;21)(q24;q22) is a rare nonrandom chromosomal aberration first described in 1989 in a pediatric patient with de novo acute myeloid leukemia (AML).[1] As a result, the *RUNX1* (runt-related transcription factor 1) gene, located at 21q22, fuses to the *CBFA2T3* (core binding factor runt domain alpha subunit 2) gene located at 16q24.[2] The RUNX1-CBFA2T3 fusion protein acts, throughout normal and neoplastic myelopoiesis, as an altered transcriptional corepressor capable of recruiting histone deacetylases and suppressing the expression of *RUNX1* target genes.[3,4]

To date, only a very few pediatric cases of t(16;21)(q24;q22) generating the *RUNX1-CBFA2T3* fusion gene have described, almost exclusively in patiens with de novo AML.[5,6]

Here we describe a case of therapy-related AML (t-AML) with t(16;21)(q24;q22) occurring in a 2-year-old after receiving chemotherapy for Askin tumor, a primitive neuroectodermal tumor of the chest wall belonging to the Ewing sarcoma (ES) family of tumors (EFT).[7] This family encompasses a number of aggressive oncofusion-driven cancers, collectively representing the second most

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common primary bone and soft tissue malignancies affecting children and adolescents.[7,8]

Given the extreme rarity of t(16;21)(q24;q22) t-AML in the pediatric setting, we also present a summary of the 4 previously described cases, which supports EFT as embodying a group of primary neoplasms associated with the development of *RUNX1-CBFA2T3*–rearranged t-AML in childhood.

## Case Report

A 2-year-old boy primarily presented with ES of the chest wall (Askin tumor) involving the fifth and sixth ribs on the right hemithorax. He received 4 courses of induction chemotherapy (EURO-E.W.I.N.G-99)[9,10] including vincristine, ifosfamide, doxorubicin, and etoposide (VIDE) and extensive surgical resection of primary neoplasm, from the fourth to the seventh rib, which were found at histopathology to be tumor uninvolved. The patient received consolidation therapy with 4 courses of vincristine, actinomycin D, and ifosfamide (VAI). The cumulative amount of each anticancer agent delivered was: vincristine, 12 mg/m$^2$; ifosfamide, 72,000 mg/m$^2$; doxorubicin, 240 mg/m$^2$; etoposide, 1,800 mg/m$^2$; actinomycin D, 6 mg/m$^2$. No radiotherapy was provided. Eighteen months after treatment completion, the patient developed sudden-onset weakness, paleness, hemorrhagic rash, and fever. At admission, he was anemic (hemoglobin, 8.9 g $\times$ 10$^9$/L) and thrombocytopenic (platelets, 10 $\times$ 10$^9$/L), with a white blood cell count of 4.3 $\times$ 10$^9$/L and 7% peripheral blasts. Bone marrow examination showed 66% of blast cells with fine chromatin, prominent nucleoli, and basophilic cytoplasm with some Auer rods, and which strongly (90%) stained for myeloperoxidase (MPO) (Supplemental Figure 1 in the online version). Blasts cells expressed CD45, CD52, CD34, CD33, HLA-DR, CD117, MPO, and CD99 while weakly staining for CD19, CD13, and CD71. ES tumor cells were absent in bone marrow. The final diagnosis was of AML with maturation (AML-M2). Cytogenetics disclosed a 46, XY, t(16;21)(q24;q22) karyotype, without trisomy 8 and without deletions/gains involving chromosomes 5 and 7, despite the common presence of these latter aberrations in t-AML.[11] He was treated with the AIEOP AML 2002/01 program regimen[12] and received consolidation therapy with haploidentical stem-cell transplantation.[13] The patient remains in ongoing complete remission at 5 years after transplantation.

The child's parents provided written informed consent on his behalf in accordance with the Declaration of Helsinki. This study is compliant with ethical standards.

Core binding factor (CBF) aberrations were analyzed by reverse transcriptase PCR (RT-PCR) with primers designed according to European BIOMED-1 Concerted Action[14] (Supplemental Tables 1 and 2 in the online version). RT-PCR analysis was negative for CBFB-MYH11 transcripts but disclosed a *RUNX1-CBFA2T1* fusion gene with an atypical amplification band (size 257 bp) (Figure 1A). Interestingly, the amplicon sized differently from PCR products (395 bp) typically described for the t(8;21)-positive reference cell line Kasumi-1.[14] Direct Sanger sequencing of this uneven PCR product detected the in-frame fusion of exon 5 of the *RUNX1* gene with exon 4 of the *CBFA2T* gene, thereby defining a t(16;21)(q24;q22) *RUNX1-CBFA2T3* based on BLAST alignment (Figure 1B-D). Bone marrow and peripheral blast underwent further screening by a multiplex quantitative RT-PCR approach able to detect more than 140 breakpoints for the 30 known leukemia-associated fusion genes (Supplemental Table 3 in the online version). Such testing confirmed RUNX1-CBFA2T3 as the only fusion transcript present in AML cells. Similarly, *NPM1* and *FLT3* D835 mutations and *FLT3* duplication were absent.

## Discussion

We describe a *RUNX1-CBFA2T3*–rearranged childhood t-AML occurring 18 months after completion of an intensive chemotherapy program, inclusive of alkylators and DNA topoisomerase II inhibitors, for a previous primitive neuroectodermal tumor of the chest wall. After receipt of chemotherapy for high-risk leukemia and allogeneic transplant, the patient experienced a durable complete remission.

Cases of t-AML harboring t(16;21)(q24;q22) are sporadic in the pediatric setting. To the best of our knowledge, only 4 cases have been so far described in the literature (Table 1).

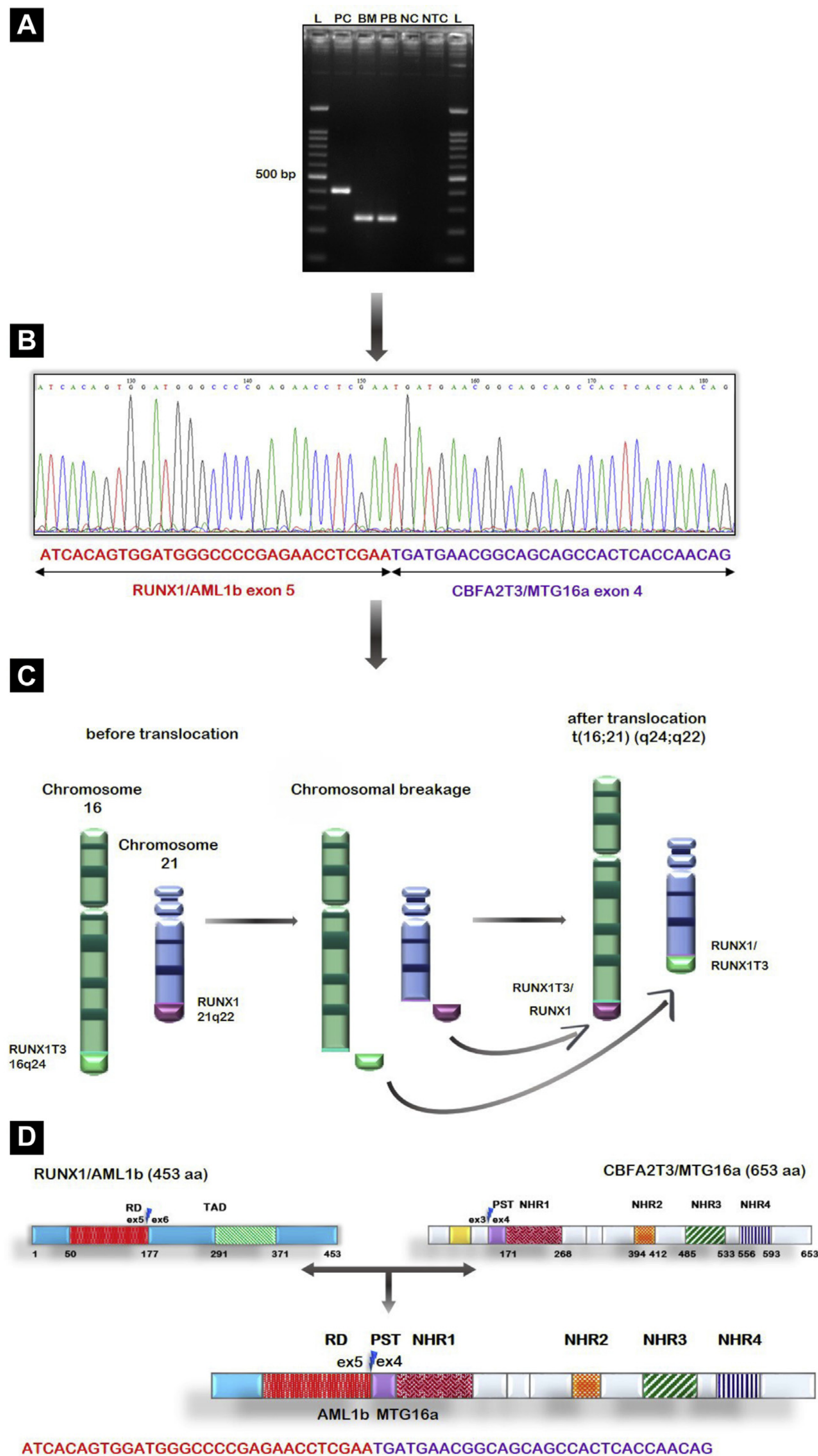
After the first two patients were described,[15,16] an additional two cases of pediatric *RUNX1-CBFA2T3*–rearranged t-AML were identified by the International Berlin–Frankfurt–Munster (I-BFM) Study Group.[6] By analyzing a data set of 1326 pediatric patients, 7 secondary AMLs were registered, 2 with *FUS-ERG* rearrangements and 5 with *RUNX1-CBFA2T3*. Of this latter group, only two cases occurred in t-AMLs. The remaining 3 patients, respectively, had a previous myelodysplastic syndrome (MDS),[6] lacked iatrogenic exposure, and did not meet the 2016 World Health Organization criteria for t-AML.[17] Overall, 3 of 4 patients with *RUNX1-CBFA2T3* AML had a preceding ES.[6,16]

The rarity of t(16;21)(q24;q22) among childhood t-AML, coupled with the significant frequency of primary EFT malignacies (4 of 5 cases; Table 1) highlights the association between this infrequent t-AML genotype and ES-related tumors. Indeed, an extensive review of secondary malignancies (SMN) in pediatric patients with ES and ES-related tumors evidenced the AML/MDS group as the single most common type of SMN, comprising up to 27% of all SMNs.[18] However, grouping AML and MDS cases together as well as the lack of availability of karyotypic details hamper a specific assessment of t(16;21)(q24;q22) t-AML frequency from these databases. Similarly, the Australian Childhood Cancer Registry has highlighted how the most common solid tumors preceding pediatric t-AML are in fact ES and rhabdomyosarcoma.[19] The increasing delivery over years of intensified chemotherapy regimens including, among other agents, alkylators, doxorubicin, and etoposide to treat EFT, represents a common determinant for t-AML development in these pediatric patients.[19,20]

Actually, 3 of 5 patients with t(16;21)(q24;q22) t-AML (Table 1) had received alkylators and DNA topoisomerase II inhibitors.[20,21] Treatment details were not individually described in the I-BFM report, but all participating groups adopted, throughout the study period, intensive chemotherapy programs including alkylators, epipodophyllotoxins, and/or anthracyclines.[6] This fact and the relatively short latency times strongly link development of these t(16;21)(q24;q22) t-AML cases to etoposide- and/or anthracycline-based treatments for primary EFTs.

Indeed, DNA topoisomerase II–associated t-AMLs display balanced translocations involving chromosomal bands 11q23 (most frequent) or 21q22 and the *MLL* or *RUNX1* genes, respectively, as

**Figure 1** PCR Analysis, Sequencing, and Graphical Representation of Fusion Transcript in t-AML. (A) Detection of t(16;21)(q24;q22) Chromosomal Translocation by RT-PCR amplification. (B) Representative Sanger sequencing of RT-PCR bands. (C) Graphical Representation of Relevant Chromosomes before (Left) and after (Right) t(16;21)(q24;q22) Translocation Formation. (D) Schematic Representation of *RUNX1* and *CBFA2T3* Genes. *RUNX1* Exon 5 is Fused in Frame to *CBFA2T3* Exons 4 and Fusion Product *RUNX1-CBFA2T3*




Abbreviations: BM = bone marrow; L = ladder; NC = negative control; NHR1-4 = *Drosophila* nervy homologous region 1-4; NTC = no template control; PB = peripheral blood; PC = positive control; PST = proline/cerine/threonine-rich region; RD = runt domain; TAD = transcriptional activation domain; t-AML = therapy-related acute myeloid leukemia.

well as t(8;21)(q22q22), t(3;21), inv(16)(p13q22), t(8;16), t(15;17)(q22,12), and t(9;22).[22,23] Furthermore, the 21q22 region typically represents a preferential target for DNA topoisomerase II inhibitors, also the result of a common hot-spot ATGCCCCAG sequence in the *RUNX1* intron 5 breakpoint.[5,24,25]

Occurrence of t-AML cases very shortly after, or even at the same time as, primary therapy for EFT has suggested that t(16;21)(q24;q22) AML may not be uniquely related to previous treatments.[11,26,27] However, searching for genetic host-related factors predisposing EFT patients to develop SMNs has been to date mostly elusive. Furthermore, while in adults the latency period before t-AML is usually on the order of years, in the pediatric setting, it appears more variable, ranging from a few months to several years.[19] In this light, although the 21q22 area is highly susceptible to translocation both in EFT and AML, and although chimeric oncogenes involved in EFT may have significant implications in the tumorigenesis of human hematopoietic disorders,[28] a pathobiologic link between the occurrence of EFT and the risk of AML development remains to be documented.

**Table 1 Clinical and Karyotypic Characteristics of Reported Cases of Childhood t-AML With t(16;21)(q24;q22)**

| Age (Years)/Sex | Primary Tumor | FAB Type | Karyotype | Cumulative Doses of CHT for Primary Tumor (mg/m$^2$) | Prior Radiotherapy | Latency (Months) | Outcome | Reference |
|---|---|---|---|---|---|---|---|---|
| 11/M | APL with PML-RARα | t-AML-M2 | 46, XY, add(12)(p13), t(16;21)(q24;q22) | ATRA, cytarabine (52,200), daunomycin (140), mitoxantrone (18), pirarubicin (83), busulfan (450), L-PAM (176), etoposide (1,850) | No | 12 | URD-BMT → ACR (20+ months) | 15 |
| 14/M | ES | t-AML-M5a | 47, XY, add(4)(q35), +8, add(16)(q24)[23]/47, XY, +8, t(16;21)(q24;q22) | Vincristine (8.5), cyclophosphamide (16,000), doxorubicin (420), ifosfamide (27,000), etoposide (2,700) | 46 Gy | 36 | CR (14 months) → died of MOF | 16 |
| < 18 | ES | NED | …t(16;21)(q24;q22)… | NED | NED | NED | NED | 6 |
| < 18 | ES | NED | …t(16;21)(q24;q22)… | NED | NED | NED | NED | 6 |
| 4/M | ES | t-AML-M2 | 46, XY, t(16;21)(q24;q22) | Vincristine (12), ifosfamide (72,000), doxorubicin (240), etoposide (1,800), actinomycin D (6) | No | 18 | RD-BMT → ACR (36+ months) | Present |

Abbreviations: ACR = alive in complete remission; APL = acute promyelocytic leukemia; ATRA = all-*trans* retinoic acid; CHT = chemotherapy; CR = complete remission; ES = Ewing sarcoma; FAB = French–American–British; L-PAM = L-phenylalanine mustard; MOF = multiorgan failure; NED = not explicitly detailed; RD-BMT = related-donor bone marrow transplant; t-AML = therapy-related acute myeloid leukemia; URD-BMT = bone marrow transplant from unrelated donor.

## Conclusion

Only 5 pediatric cases of secondary AML harboring t(16;21)(q24;q22) *RUNX1-CBFA2T3* have hitherto been described; notably, 4 of them had ES as a primary neoplasm. Previous exposure to DNA topoisomerase II inhibitors represents the most substantiated causal mechanism for t-AML development. The leukemogenic potential of ES-associated fusion genes and their homologs[29] may stimulate further studies addressing whether EFT may somehow provide a biologic setup favoring development of t-AML specifically harboring the *RUNX1-CBFA2T3* fusion gene.

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## Disclosure

The authors have stated that they have no conflict of interest.

## Supplemental Data

Supplemental tables and figure accompanying this article can be found in the online version at https://doi.org/10.1016/j.clml.2020.05.020.

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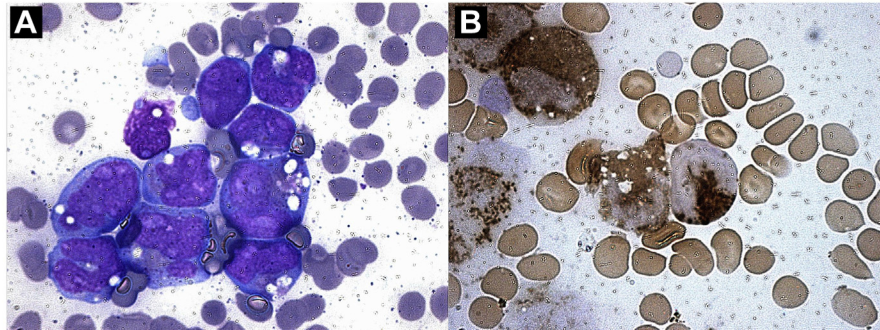
**Supplemental Figure 1** Bone Marrow Aspirate Smears Showing Myeloblasts and Cytoplasmic Granules. (A) Evident are Increased Numbers of Myeloblasts With Irregular, Large Indented Nuclei and Fine Chromatin Pattern, Prominent Nucleoli, basophilic Cytoplasm, With some Auer Rods (Wright-Giemsa stain, Original Magnification ×100). (B) Myeloperoxidase-Positive Cytoplasmic Granules in Blast Cells (Dark brown Dots) (Cytochemical stain, Original Magnification ×100)




**Supplemental Table 1** RT-PCR Analysis of t(8;21)(q22;q22) with *AML1-MTG8* Fusion Gene[14]

| Primer Code | 5′ Position (Size in bp) | Sequence (5′-3′) | Positive Control | Size of PCR Product (bp) |
|---|---|---|---|---|
| AML1-Forward FR[a] | 884 (21) | CTACCGCAGCCATGAAGAACC | Kasumi-1 | 395 |
| MTG8-Reverse FR[a] | 495 (21) | AGAGGAAGGCCCATTGCTGAA | | |
| AML1-Forward N[b] | 920 (22) | ATGACCTCAGGTTTGTCGGTCG | | 260 |
| MTG8-Reverse N[b] | 396 (22) | TGAACTGGTTCTTGGAGCTCCT | | |

Abbreviation: RT-PCR = reverse transcriptase PCR.
[a]First round PCR.
[b]Nested PCR.

**Supplemental Table 2** **PCR Thermal Cycling Conditions.[14] (van Dongen JJ, Macintyre EA, Gabert JA, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia*. 1999; 13: 1901-28)**

| Step | Temperature (°C) | Duration |
| --- | --- | --- |
| First-round RT-PCR thermal cycling conditions | | |
| 1 | 94 | 5 minutes |
| 2 × 35 cycles | 94 | 30 seconds |
| | 62 | 60 seconds |
| | 72 | 60 seconds |
| 3 | 72 | 10 minutes |
| Nested PCR thermal cycling conditions | | |
| 1 | 94 | 5 minutes |
| 2 × 30 cycles | 94 | 30 seconds |
| | 60 | 60 seconds |
| | 72 | 60 seconds |
| 3 | 72 | 10 minutes |

PCR products were analyzed by 2% agarose gel electrophoresis at 150 V for 1 hour.
Abbreviation: RT-PCR = reverse transcriptase PCR.

**Supplemental Table 3** **Additional 30 Leukemia Fusion Genes Examined by Multiplex Quantitative RT-PCR**

| |
| --- |
| *MLL-AF9* |
| *MLL-AF4* |
| *MLL-MLLT1* |
| *MLL-MLLT10* |
| *PML-RARA* |
| *TEL-AML1* |
| *BCR-ABL1* |
| *CBFB-MYH11* |
| *AML1-MTG8* |
| *E2A-PBX1* |
| *STIL-TAL1* |
| *AML1-EVI1* |
| *FIP1L1-PDGFRA* |
| *MLL-SEPT6* |
| *MLL-AF17* |
| *MLL-AF1P* |
| *DEKNUP214* |
| *MLL-ELL* |
| *MLL-MLLT11* |
| *MLL-MLLT4* |
| *SET-NUP214* |
| *FUS-ERG* |
| *NPM1-RARA* |
| *TEL-ABL1* |
| *E2A-HLF* |
| *TEL-PDGFRB* |
| *ZBTB16-RARA* |
| *AML1-EAP* |
| *NPM1-MLF1* |
| *AML1-CBFA2T3* |

Abbreviation: RT-PCR = reverse transcriptase PCR.