

## Case Report

# Mutations within the Activation Loop Domain of FLT3 in Two Pediatric Patients with Refractory Infant Acute Myeloid Leukemia

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## Keywords

FLT3-TKD mutation · Infant acute myeloid leukemia · FLT3 inhibition · Pediatric oncology

## Abstract

Approximately 24% of all pediatric acute myeloid leukemia (AML) cases have mutations in the FMS-like tyrosine kinase 3 (*FLT3*) receptor gene. FLT3-TKD point mutations are rare in pediatrics and often occur in younger patients and in combination with 11q23 abnormalities. There is a paucity of data related to their prognostic implications in children. We describe 2 pediatric patients with FLT3-activating mutations as a feature of their AML. Both were diagnosed in infancy. The first experienced induction failure and had refractory disease without expression of FLT3-TKD mutation on subsequent bone marrow evaluations. His disease also harbored a KMT2A-PICALM gene rearrangement. He died of invasive fungal disease nine months after diagnosis. The second had a post-induction remission but developed swelling of the left calcaneus shown on biopsy to be a myeloid sarcoma positive for a new BRAF V600E mutation in addition to his known KMT2A rearrangement but without FLT3-TKD mutation. Despite multiple courses of therapy including BRAF/MEK-inhibition, he died of progressive disease nine months after diagnosis. FLT3 inhibition was not utilized in either patient as studies have largely focused on its role in internal tandem duplication (ITD) mutations and because the mutation was no longer detectable in either patient on subsequent evaluation. However, these cases add to the suggestion that these mutations confer a worse prognosis in pediatric AML patients.

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## Introduction

Approximately 24% of all pediatric acute myeloid leukemia (AML) cases have mutations in the FMS-like tyrosine kinase 3 (*FLT3*) receptor gene [1]. Activating mutations lead to constitutive kinase activity, uncontrolled hemopoietic proliferation, and inhibition of cell differentiation and apoptosis. This most commonly occurs as the result of internal tandem duplications of the *FLT3* gene (FLT3-ITD), in which duplicated DNA sequences are inserted into the gene, altering the juxtamembrane domain to allow for signaling activation without binding of the FLT3 ligand. A single series suggests that ITD mutations portend a poor prognosis, especially among patients with higher allelic ratios [1,2]. FLT3-tyrosine kinase domain (TKD) mutations involve a single point mutation in the activating loops of the TKD that also leads to constitutive kinase activity [3]. FLT3-TKD point mutations are rare in pediatrics, having previously been reported in about 7% of all pediatric AML patients [4]. They occur most commonly in younger patients and in combination with 11q23 abnormalities. In contrast to an association with poor prognosis in adults, FLT3-TKD mutations have not been shown to be a risk factor for induction failure or relapse in pediatric patients [4, 5]. However, there is a paucity of data related to the prognostic value of FLT3-TKD in pediatric patients [6]. We aim to add to the limited literature on this topic a description of two cases of infant AML with FLT3-TKD mutations and their unfavorable clinical courses.

## Case Presentation

### Case 1

A 4-month-old, previously healthy, male born at full term presented to the emergency department with fevers and bruising. His complete blood count was remarkable for a white blood cell count of  $506 \times 10^9/L$ , hemoglobin of 7.5 g/dL, and platelet count of  $35 \times 10^9/L$ . Bone marrow evaluation confirmed the diagnosis of AML with 30% blasts. He had central nervous system (CNS) 2 disease. Chromosomal analysis revealed a karyotype of 47,XY,del(11)(q14q23). Foundation Medicine reported a KMT2A-PICALM gene rearrangement. In addition to an 11q23 (KMT2A) rearrangement, a FLT3-TKD-activating mutation at codon D835 was detected by next-generation sequencing in 21.6% of all cells (Table 1).

The patient received standard induction chemotherapy with cytarabine, daunorubicin, and etoposide. He had persistent CNS involvement throughout his course despite intensification with twice weekly intrathecal cytarabine for a total of 13 lumbar punctures. Bone marrow examination at the time of first hematologic recovery revealed persistence of his AML with 41% blasts. KMT2A rearrangement and FLT3-TKD mutation remained detectable by next-generation sequencing (Table 2). A second induction attempt with mitoxantrone and cytarabine resulted in persistent disease with 8% blasts in the marrow and continued detection of blasts in the cerebrospinal fluid. He developed new skin lesions which were biopsy-proven leukemia cutis. Despite multiple attempts to achieve remission, his disease remained refractory, and he subsequently died at 13 months of age of invasive fungal disease.

### Case 2

A 9-month old, previously healthy, male born at full term presented to his pediatrician with fevers and scalp lesions. Ultrasound showed two lytic lesions within the skull. Due to concern for solid tumor metastases, a chest X-ray and abdominal ultrasound were performed. The chest X-ray was clear, and the ultrasound demonstrated a  $5 \times 4 \times 2$  cm left suprarenal mass. Complete blood count showed evidence of a mild normocytic anemia with 3% blasts noted. Bone marrow examination showed effacement of marrow by blasts of monocytic

**Table 1.** Features in two cases with FLT3-TKD

Patient	Age, months	Sex	FAB	FLT3-TKD
1	4	M	M5	D835Y (Asp/Tyr)
2	9	M	M5	N841K (Asn/Lys)

**Table 2.** Serial molecular assessment of FLT3-TKD in the setting of refractory disease

Patient	At diagnosis	Following Cycle 1	Following Cycle 2	Following Cycle 3	Following Cycle 4
1	21.6% of reads	0.1% cells with FLT3-TKD	FLT3-TKD not detected	FLT3-TKD not detected	N/A
2	29.6% of reads	FLT3-TKD not detected	N/A	FLT3-TKD not detected	FLT3-TKD not detected

lineage consistent with AML. Scalp biopsy revealed similar leukemic infiltrates. Chromosomal analysis showed a cryptic KMT2A rearrangement, with a karyotype of 47,XY,+8[7]/50,XY,+der(?)(?;8)(?;q24.2)+der(?)+der(?)+der(?)[6]/46,XY[7] with four copies of MYC. Next-generation sequencing detected a FLT3-TKD-activating mutation at codon N841 (Table 1). He had no CNS involvement.

The patient received standard induction chemotherapy with cytarabine, daunorubicin, and etoposide. Following this cycle of chemotherapy, his bone marrow examination demonstrated remission by morphology, FISH, and flow-based minimal residual disease assessment (Hematologics, Inc., Seattle, WA). On recovery from induction chemotherapy, he developed swelling and pain of his left calcaneus evaluated by MRI, which was presumed to be osteomyelitis. Given evidence of remission, he continued with standard chemotherapy and intravenous antibiotics. Calcaneus symptoms improved but worsened with subsequent hematologic recovery. Marrow examination and chromosomal analysis following a second cycle of cytarabine, daunorubicin, and etoposide again demonstrated no evidence of disease.

He continued with a cycle of high-dose cytarabine and etoposide as per Intensification IA of Children's Oncology Group AAML1031. Given the lack of response with broad-spectrum antibiotics, the calcaneus was ultimately biopsied and found to be a myeloid sarcoma which was positive for a new BRAF V600E mutation in addition to his known KMT2A rearrangement; FLT3-TKD mutation was not detectable from the calcaneal biopsy (Table 2). He then received Capizzi-style cytarabine, Erwinia asparaginase, and gemtuzumab, following which he had persistence of the calcaneal chloroma and developed soft tissue extension and extensive FDG-PET-avid adenopathy. Reinduction was attempted with a mitoxantrone and cytarabine regimen plus 2,400 cGy calcaneal radiotherapy. He had improvement of the calcaneal lesion but progressive adenopathy. Disease remained refractory to topotecan, vinorelbine, thiotepa, and clofarabine (TVTC), compassionate use of venetoclax with decitabine, and combined BRAF/MEK inhibition. FLT3 inhibition was not given. The patient died at 18 months of age of progressive disease.

## Discussion

Here, we describe two pediatric patients with FLT3-activating mutations as a feature of their AML. Because FLT3-TKD mutations are present in only 7% of all pediatric AML cases, the prognostic value and role of FLT3 inhibition in the setting of FLT3-TKDs is limited.

Meshinchi et al. [4] reported that FLT3-TKDs have improved outcomes in the pediatric population compared to other activating mutations, namely FLT3-ITD mutations, c-kit, and ras mutations, consistent with findings reported in adult patients. Multiple mutations within the

activation loop of FLT3 have been described in adult literature, with D835Y being the most common [7, 8]. These have been associated with poor disease-free survival in younger adults [5]. A prior pediatric series found three activating mutations in codon 835 of FLT3 among 91 patients; the clinical outcomes were variable [9]. Mutations in residue N841 have been described in adult AML; however, our case is the first reported in pediatrics. It has been suggested that N841 may be particularly sensitive to small-molecule inhibitors [10], but the clinical significance of specific point mutations in pediatric AML has yet to be described. It is unknown whether this specific FLT3-TKD mutation was responsible for the refractory nature of our patients' disease.

Additional mutations in our patients may have contributed to their leukemogenesis as well. Patient 1 had a KMT2A-PICALM rearrangement. This gene rearrangement is thought to contribute to leukemogenesis via disruptions of normal endocytosis and vesicle transport [11]. Similarly, KMT2A-AF9 fusion may have contributed to the leukemogenesis in patient 2. The development of a BRAF mutation in patient 2 most likely represents clonal evolution of the patient's leukemia. In each case, the FLT3-TKD mutation did not re-emerge after becoming undetectable. In adults, FLT3-TKD mutations are often not preserved at the time of relapse [3]. The disappearance of FLT3-TKD in patient 2 and the development of a BRAF V600E mutation in patient 2 suggests clonal evolution of the patient's leukemia. BRAF V600E mutations are uncommon in AML; in a single case series of 399 adult and pediatric patients, only four patients had V600E mutations [12]. Similar to patient 2, two of the four cases in the series developed extramedullary involvement, and the myeloid leukemia was of monocytic lineage [12]. BRAF inhibition has been employed in adult hairy cell leukemia patients [13], but has yet to be well described in AML.

Pediatric studies evaluating the role of FLT3 inhibition have largely focused on ITD mutations [14–16]. The therapeutic impact of FLT3 inhibitors in FLT3-TKD-mutated AML is limited. The addition of a FLT3 inhibitor was considered for each patient. However, FLT3-TKD was no longer detectable in either patient when the patients were found to have refractory disease. Therefore, neither received FLT3-directed therapy. Upfront FLT3 inhibition in adult patients, regardless of FLT3 mutation, leads to improved overall survival and event-free survival [17]. The Children's Oncology Group AML committee tested FLT3 inhibition in pediatric patients with FLT3-ITD high allelic ratios prospectively as part of AAML1031, and these data are not yet published. Furthermore, the benefit of FLT3 inhibition in pediatric patients with FLT3-TKD mutations is limited to a single report.

Genetic sequencing in cases of AML, especially among younger patients, will continue to identify novel and potentially targetable mutations and contribute to the small but growing body of literature in the field of pediatric AML [18]. As the genetic landscape of infant AML evolves, there will be improved understanding of the prognostic value of FLT3-TKDs and the utility of FLT3 inhibition.

### Statement of Ethics

The study protocol was approved by the Lifespan Institutional Review Board. Written consent could not be obtained from the 2 patients' families at the time of this analysis; however, requirement of informed consent was waived by the Review Board as the analysis would not directly influence or change the care of these subjects.

### Disclosure Statement

The authors have no conflicts of interest to declare.

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None.

## Author Contributions

All authors conceived and designed the analysis. N.M. collected the data. N.M. and R.E.M.-Y. wrote the first draft of the paper. All authors contributed to the data analysis and revisions. All authors approved the final manuscript.

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