UCSF

UC San Francisco Previously Published Works

Title

NUP98-NSD1 Driven MDS/MPN in Childhood Masquerading as JMML.

Permalink

<https://escholarship.org/uc/item/62h3f1fm>

Journal

Journal of pediatric hematology/oncology, 43(6)

ISSN

1077-4114

Authors

Behnert, Astrid
Lee, Alex G
Young, Elizabeth P
et al.

Publication Date

2021-08-01

DOI

10.1097/mph.0000000000001913

Peer reviewed

NUP98-NSD1 Driven MDS/MPN in Childhood Masquerading as JMML

Astrid Behnert, MD,*† Alex G. Lee, PhD,*† Elizabeth P. Young, MD,*
 Marcus R. Breese, PhD,*† Stanley G. Leung, BA,*† Inge Behroozfard, BS,*
 Maria Maruffi, MD,‡ E. Alejandro Sweet-Cordero, MD,*†
 Christopher C. Dvorak, MD,*† Julia Chu, MD,* and Elliot Stieglitz, MD*†

Summary: Overlapping myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are clonal hematopoietic disorders with features of myelodysplasia and myeloproliferation. The only well-characterized MDS/MPN in children is juvenile myelomonocytic leukemia, an aggressive disorder of infants and toddlers. The biochemical hallmark of this disease is hyperactivation of the Ras/MAPK signaling pathway caused by mutations in Ras pathway genes in more than 90% of patients. Translocations involving receptor tyrosine kinases have been identified in rare cases. Here, we report a 2-year-old patient who presented with MDS/MPN driven by a cytogenetically cryptic *NUP98-NSD1* fusion, a translocation thought to exclusively occur in patients with acute myeloid leukemia.

Key Words: MDS/MPN, JMML, NUP98-NSD1

(*J Pediatr Hematol Oncol* 2020;00:000–000)

Myelodysplastic/myeloproliferative neoplasms (MDS/MPN) are clonal hematopoietic disorders characterized by aberrant proliferation with increased immature cells in the peripheral blood as well as varying degrees of dysplasia and cytopenias. These disorders have an increased risk of progression to acute myeloid leukemia (AML) and are further divided into atypical chronic myeloid leukemia, chronic myelomonocytic leukemia, juvenile myelomonocytic leukemia (JMML), and a fourth category, MDS/MPN-unclassifiable, which is reserved for myeloid neoplasms not meeting diagnostic criteria for any of the other 3.¹ MDS/MPN is a rare diagnosis in children, occurring in ~1 to 4 cases per million, best exemplified by JMML, a frequently fatal disease in early childhood.² Children with JMML typically present with fever, anemia, thrombocytopenia, and leukocytosis with peripheral monocytosis.² Most children also present with splenomegaly, while others develop it within a few weeks of diagnosis.³ Bone marrow aspirate typically reveals varying degrees of abnormal myelo- or megakaryopoiesis but must display fewer than 20%

blasts to rule out AML. Translocations have been reported in JMML^{4,5} but the majority of patients with JMML (>95%) have somatic or germline mutations detected in genes encoding proteins signaling in the RAS/MAPK pathway.⁶ Most patients with JMML receive allogeneic hematopoietic cell transplantation (HCT), which currently remains the only curative treatment option.² Here, we report a patient who was suspected of having JMML but was found to have a cryptic *NUP98-NSD1* fusion detected on RNA sequencing (RNASeq) that has not been previously reported in MDS/MPN.

CASE REPORT

A 2-year-old girl presented after 6 months of progressive symptoms including fatigue, pallor, and gingival hyperplasia, and was found to have severe anemia, thrombocytopenia, leukocytosis (85.2 K/ μ L with 20% monocytes) and elevated age-adjusted hemoglobin F (37.1%). Bone marrow aspirate revealed 5% myeloid blasts, increased promonocytes, dysplastic features, and absence of *BCR/ABL*. Biopsy of a scalp lesion revealed a myelomonocytic infiltrate, consistent with leukemia cutis. Abdominal ultrasound was negative for hepatosplenomegaly. Cytogenetic analysis showed a single abnormal cell with a 44,X,-X,del(9)(q13q22),-16[1],46,XX[20] karyotype. *FLT3*-ITD, *CEBPA*, and *NPM1* were tested using next generation sequencing and were all normal. An institutional DNA sequencing panel detected a mutation in *NRAS* p.Gly12Val at an allele frequency of 8%, a buccal germline sample was negative. Diagnosis of JMML was suspected although the patient did not have splenomegaly at time of diagnosis and only a subclonal *RAS* mutation was detected. A presumptive diagnosis of MDS/MPN-unclassifiable requiring HCT was made. Granulocyte-macrophage-colony-stimulating factor hypersensitivity testing was not performed.

METHODS

The patient's guardians provided informed consent to this study in accordance with the Declaration of Helsinki. DNA and RNA were extracted using standard methods from buccal and bone marrow mononuclear cells. RNASeq and whole genome sequencing were performed. For additional details, see Supplemental Methods, Supplemental Digital Content 1 (<http://links.lww.com/JPHO/A394>).

RESULTS

The patient was treated with fludarabine and cytarabine to reduce her disease burden before HCT.⁷ After 1 cycle of chemotherapy, the *NRAS* mutation was no longer detectable and cytogenetic analysis was normal. However, flow cytometry revealed a CD34⁺ myeloblast population of ~0.5% with a subset showing immunophenotypic abnormalities with dim partial CD7 and CD15 expression and moderate to bright CD123 expression. After conditioning with busulfan, cyclophosphamide, melphalan, and rabbit antithymocyte globulin,⁸ she received

Received for publication May 11, 2020; accepted June 28, 2020.

From the *Department of Pediatrics, Benioff Children's Hospital; †Helen Diller Comprehensive Cancer Center, University of California San Francisco, San Francisco; and ‡Department of Pediatric Subspecialty, Kaiser Permanente, Oakland, CA.

Supported by the Frank A. Campini Foundation (E.S.) and the German Cancer Aid (A.B.).

The authors declare no conflict of interest.

Reprints: Elliot Stieglitz, MD, UCSF, Stieglitz Lab 1450 3rd St, Box 3112, San Francisco, CA 94158 (e-mail: elliott.stieglitz@ucsf.edu).

Supplemental Digital Content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's website, www.jpheonline.com.

Copyright © 2020 Wolters Kluwer Health, Inc. All rights reserved.

FIGURE 1. A, Reverse transcription polymerase chain reaction (RT-PCR) product of ~574 base pair on 1.5% agarose gel (MWM, molecular weight marker; lane 1: 15 μ L of RT-PCR product loaded; lane 2: 5 μ L of RT-PCR product loaded). B, Schematic illustration of *NUP98-NSD1* fusion. [full color online](#)

allogeneic HCT from a 12/12 human leukocyte antigen-matched unrelated donor. Shortly after HCT, research-grade RNA sequencing using diagnostic material returned, demonstrating a *NUP98-NSD1* gene fusion. Direct sequence analysis revealed an in-frame gene fusion between the *NUP98* gene on exon 12 (chr11:3744509) and *NSD1* on exon 6 (chr5:177235821) (Fig. 1B). Reverse transcription polymerase chain reaction (RT-PCR) was used to validate this finding using primers flanking *NUP98* and *NSD1* genes, resulting in a band of ~574 base pairs (Fig. 1A). The expected sequence was confirmed by Sanger sequencing. Principal component analysis of RNASeq data from patients with JMML, AML, and acute lymphoblastic leukemia showed that this patient clustered closer to other patients with AML compared to those with JMML (Fig. 2).

Whole genome sequencing from diagnosis also confirmed the presence of the *NRAS* mutation and absence of a *FLT3*-ITD but did not reveal any additional mutations. Clinical-grade RT-PCR was then used to confirm the *NUP98-NSD1* fusion using diagnostic material which allowed for posttransplant monitoring of transcript levels.

The patient's treatment course was complicated by veno-occlusive disease and transplant-associated thrombotic microangiopathy, which were successfully treated with defibrotide and eculizumab. The patient is currently alive and well with absence of *NUP98-NSD1* fusion expression by RT-PCR 5 months after HCT (Fig. 3). However, the patient continues to evidence mixed chimerism in her CD3⁺ cells despite rapid taper of tacrolimus. In an effort to achieve full

FIGURE 2. Principal component analysis of RNASeq data from patients with acute lymphoblastic leukemia (n = 878, highlighted in red), patients with acute myeloid leukemia (n = 538, highlighted in yellow), and patients with juvenile myelomonocytic leukemia (n = 20, highlighted in blue). Our patient with *NUP98-NSD1* MDS/MPN is indicated by a black arrow. [full color online](#)

FIGURE 3. Schematic timeline of patient's clinical course. Timepoints shown are relative to HCT. *NUP98-NSD1 expression showed a log reduction of >4 from baseline. Ara-C indicates cytarabine; AZA, azacitidine; DLI, donor lymphocyte infusion; Flu, fludarabine; HCT, hematopoietic cell transplantation; MAF, mutant allele frequency.

donor chimerism, the patient has received 2 cycles of azacitidine followed by donor lymphocyte infusions but remains mixed to date (Fig. 3).⁹

DISCUSSION

NUP98-NSD1 is the result of a chromosomal translocation (t(5;11)(q35;p15.5) that generates a fusion between the N-terminus of nucleoporin 98 (NUP98) and the C-terminus of the nuclear receptor binding SET domain protein 1 (NSD1). Because both NUP98 and NSD1 are located in the telomeric region of chromosomes 11 and 5, respectively, fusions involving these 2 genes are cryptic by conventional karyotyping. NUP98-NSD1 is the most frequent gene fusion in cytogenetically normal AML,¹⁰ but has not yet been reported in MDS, MPN, or overlapping MDS/MPN including JMML. Patients with NUP98-NSD1 AML have significantly higher white blood cell count at diagnosis and frequent FAB-M4/M5 morphology compared to patients without the fusion.¹¹ The presence of NUP98-NSD1 is also reported to be a poor prognostic marker in pediatric and adult AML.^{11,12} However, it was later shown that pediatric patients with NUP98-NSD1 fusions frequently have cooccurring FLT3-ITD alterations and while the cooccurrence in patients is associated with lower overall and event-free survival, patients with NUP98-NSD1 alone have outcomes similar to patients without the fusion.¹³

To date, 31 different fusion partners of NUP98 have been identified and the resulting fusion products are thought to lead to epigenetic dysregulation of different leukemia-associated genes including HOXA7 and HOXA9.^{11,14} The NUP98-NSD1 fusion has been shown to be sufficient to cause malignant transformation of normal hematopoietic cells.¹⁴ Additional somatic mutations in other genes including NRAS and more commonly FLT3-ITD have been identified and are described as secondary events promoting malignant transformation.^{15,16} A variety of NUP98 fusions have been reported in MDS.¹⁷ One previous case report describes a NUP98-HOXA11 fusion in a 3-year-old girl with JMML who also had a heterozygous mutation in NRAS p. Gly13Asp.¹⁸ However, the NUP98-NSD1 gene fusion detected in our patient has only been reported in AML.

This case highlights the benefit of RNASeq in pediatric patients who present with diagnostic uncertainty and the need for rapid turn-around-times in clinical laboratories. Earlier identification of this fusion would have altered the

clinical management of this patient. With knowledge of the NUP98-NSD1 fusion, chemotherapy would have been continued until RT-PCR was negative, as achieving a molecular remission before transplant has been associated with improved outcomes in both JMML and AML.^{19,20} In addition, it would have prompted use of a less intensive myeloablative conditioning regimen, which may have spared some of the patient's post-HCT toxicities. Lastly, this finding was of benefit by offering the ability to track the patient's disease status more accurately post-HCT using RT-PCR. In summary, this represents the first case of a NUP98-NSD1 fusion in a pediatric MDS/MPN patient and expands the spectrum of fusions in this category.

REFERENCES

1. Clara JA, Sallman DA, Padron E. Clinical management of myelodysplastic syndrome/myeloproliferative neoplasm overlap syndromes. *Cancer Biol Med.* 2016;13:360–372.
2. Chang TY, Dvorak CC, Loh ML. Bedside to bench in juvenile myelomonocytic leukemia: insights into leukemogenesis from a rare pediatric leukemia. *Blood.* 2014;124:2487–2497.
3. Niemeyer CM, Arico M, Basso G, et al. Chronic myelomonocytic leukemia in childhood: a retrospective analysis of 110 cases. European Working Group on Myelodysplastic Syndromes in Childhood (EWOG-MDS). *Blood.* 1997;89:3534–3543.
4. Byrgazov K, Kastner R, Dworzak M, et al. A novel fusion gene NDEL1-Pdgfrb in a patient with JMML with a new variant of TKI-resistant mutation in the kinase domain of PDGFRβ. *Blood.* 2014;124:613.
5. Chao AK, Meyer JA, Lee AG, et al. Fusion driven JMML: a novel CCDC88C-FLT3 fusion responsive to sorafenib identified by RNA sequencing. *Leukemia.* 2020;34:662–666.
6. Murakami N, Okuno Y, Yoshida K, et al. Integrated molecular profiling of juvenile myelomonocytic leukemia. *Blood.* 2018;131:1576–1586.
7. Stieglitz E, Ward AF, Gerbing RB, et al. Phase II/III trial of a pre-transplant farnesyl transferase inhibitor in juvenile myelomonocytic leukemia: a report from the Children's Oncology Group. *Pediatr Blood Cancer.* 2015;62:629–636.
8. Dvorak CC, Satwani P, Stieglitz E, et al. Disease burden and conditioning regimens in ASCT1221, a randomized phase II trial in children with juvenile myelomonocytic leukemia: a Children's Oncology Group study. *Pediatr Blood Cancer.* 2018; 65:e27034.
9. Horn B, Petrovic A, Wahlstrom J, et al. Chimerism-based pre-emptive immunotherapy with fast withdrawal of immunosuppression and donor lymphocyte infusions after allogeneic stem cell

- transplantation for pediatric hematologic malignancies. *Biol Blood Marrow Transplant*. 2015;21:729–737.
10. Cerveira N, Correia C, Dória S, et al. Frequency of NUP98-NSD1 fusion transcript in childhood acute myeloid leukaemia. *Leukemia*. 2003;17:2244–2247.
  11. Hollink IHIM, van den Heuvel-Eibrink MM, Arentsen-Peters STCJM, et al. NUP98/NSD1 characterizes a novel poor prognostic group in acute myeloid leukemia with a distinct HOX gene expression pattern. *Blood*. 2011;118:3645–3656.
  12. Thol F, Köhling B, Hollink IHI, et al. Analysis of NUP98/NSD1 translocations in adult AML and MDS patients. *Leukemia*. 2013;27:750–754.
  13. Thanasopoulou A, Tzankov A, Schwaller J. Potent co-operation between the NUP98-NSD1 fusion and the FLT3-ITD mutation in acute myeloid leukemia induction. *Haematologica*. 2014;99:1465–1471.
  14. Wang GG, Cai L, Pasillas MP, et al. NUP98-NSD1 links H3K36 methylation to Hox-A gene activation and leukaemogenesis. *Nat Cell Biol*. 2007;9:804–812.
  15. Shiba N, Ohki K, Kobayashi T, et al. High PRDM16 expression identifies a prognostic subgroup of pediatric acute myeloid leukaemia correlated to FLT3-ITD, KMT2A-PTD, and NUP98-NSD1: the results of the Japanese Paediatric Leukaemia/Lymphoma Study Group AML-05 trial. *Br J Haematol*. 2016;172:581–591.
  16. Lavallée VP, Lemieux S, Boucher G, et al. Identification of MYC mutations in acute myeloid leukemias with NUP98-NSD1 translocations. *Leukemia*. 2016;30:1621–1624.
  17. Slape C, Lin YW, Hartung H, et al. NUP98-HOX translocations lead to myelodysplastic syndrome in mice and men. *J Natl Cancer Inst Monogr*. 2008;39:64–68.
  18. Mizoguchi Y, Fujita N, Taki T, et al. Juvenile myelomonocytic leukemia with t(7;11)(p15;p15) and NUP98-HOXA11 fusion. *Am J Hematol*. 2009;84:295–297.
  19. Candoni A, de Marchi F, Zannier ME, et al. High prognostic value of pre-allogeneic stem cell transplantation minimal residual disease detection by WT1 gene expression in AML transplanted in cytologic complete remission. *Leukemia Res*. 2017;63:22–27.
  20. Hecht A, Meyer J, Chehab FF, et al. Molecular assessment of pretransplant chemotherapy in the treatment of juvenile myelomonocytic leukemia. *Pediatr Blood Cancer*. 2019;66:e27948.