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Fusion driven JMML: a novel *CCDC88C–FLT3* fusion responsive to sorafenib identified by RNA sequencing

Alexander K. Chao¹, Julia A. Meyer¹, Alex G. Lee¹, Anna Hecht¹, Theodore Tarver², Jessica Van Ziffle³, Ashley K. Koegel¹, Carla Golden⁴, Benjamin S. Braun^{1,5}, E. Alejandro Sweet-Cordero^{1,5}, Catherine C. Smith^{2,5}, Christopher C. Dvorak^{1,5}, Mignon L. Loh^{1,5}, Elliot Stieglitz^{1,5}

¹Department of Pediatrics, Benioff Children's Hospital, University of California, San Francisco, San Francisco, CA, USA

²Division of Hematology/Oncology, Department of Medicine, University of California, San Francisco, San Francisco, CA, USA

³Department of Pathology, University of California, San Francisco, San Francisco, CA, USA

⁴Department of Pediatrics, Benioff Children's Hospital, University of California, San Francisco, Oakland, CA, USA

⁵Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, San Francisco, CA, USA

To the Editor

Juvenile myelomonocytic leukemia (JMML) is an aggressive myeloproliferative neoplasm of childhood with nearly half of all patients relapsing within 3 years [1, 2]. More than 90% of JMML patients present with initiating mutations *in NF1, KRAS, NRAS, RRAS, RRAS2, CBL, and PTPN11*, all leading to hyperactivation of the Ras pathway [3]. Patients with additional molecular alterations including cytogenetic abnormalities, and in particular, point mutations in *SETBP1* have inferior outcomes [4]. At present, hematopoietic stem cell transplantation (HSCT) is the only curative therapy, with the primary cause of death being relapse or progression to acute myeloid leukemia (AML) [5]. Recently, JMML patients who did not display Ras pathway mutations were found to have *ALK* and *ROS1* fusions that were sensitive to ALK inhibition [6]. Given the poor overall survival of JMML patients even after intensive treatments such as HSCT, opportunities to implement precision medicine should be

[†]Elliot Stieglitz, elliott.stieglitz@ucsf.edu.

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explored in this disease. Here, we present a JMML patient who failed to respond to conventional cytotoxic chemotherapy and was found to have a novel *CCDC88C-FLT3* fusion driving the patient's leukemia that responded to FLT3 inhibition with sorafenib. Of note, *FLT3* fusions and internal tandem duplication (ITD)s give rise to myeloproliferative disorders in mouse models but have yet to be reported in pediatric patients [7].

DNA and RNA were extracted using standard methods from bone marrow, spleen, and peripheral blood mononuclear cells obtained at various time points through the clinical course. Approvals for these studies were obtained from the University of California San Francisco (UCSF) Committee on Human Research. The guardians provided informed consent in accordance with the Declaration of Helsinki.

For additional details, see Supplemental Methods, available on the Leukemia website. All relevant data used in this study are available for download from Synapse (<https://doi.org/10.7303/syn18913127>).

A 20-week-old boy presented with hepatosplenomegaly, an elevated white blood count (WBC) of 92 K/ μ l with 60% monocytes, and leukoerythroblastosis on peripheral blood smear. A diagnostic bone marrow aspirate revealed <5% blasts, absence of *BCR/ABL1*, and no known JMML mutations. The patient's peripheral blood mononuclear cells were hypersensitive to the cytokine granulocyte-macrophage colony-stimulating factor (Fig. 1a). The patient was treated with fludarabine and high-dose cytarabine per our institutional standard of care in an attempt to reduce the disease burden prior to HSCT [1]. However, the patient was refractory to chemotherapy and experienced worsening hepatosplenomegaly, eventually requiring splenectomy. During these therapies, the patient's clinical status continued to deteriorate and he was not an eligible candidate for HSCT (Fig. 1b).

An institutional DNaseq panel assaying 480 cancer relevant genes and RNA-Seq both revealed an inframe *CCDC88C-FLT3* fusion (Fig. 1c). Although *FLT3* mutations are found in both adult and pediatric AML, *FLT3* mutations have previously been reported as rare in JMML, and no *FLT3* fusions have ever been reported in a pediatric malignancy. Given the patient's lack of response to cytotoxic chemotherapy and because he was deemed ineligible for HSCT, sorafenib, an oral FLT3 inhibitor was identified as a potential tolerable and targeted therapy. Sorafenib is FDA approved for adults with hepatocellular and renal cell carcinoma and has been shown to effectively treat a *FLT3-ETV6* fusion in an adult with a myeloproliferative fusion [8]. Prior to initiating sorafenib, we performed a bone marrow aspirate which revealed a t(13;14)(q12;q32) translocation consistent with the *CCDC88C-FLT3* fusion, as well as monosomy 7.

To determine whether the *CCDC88C-FLT3* fusion protein was oncogenic, we transduced the murine pro-B cell line Ba/F3, which is well established for studying *FLT3* alterations in myeloid malignancies with viral constructs containing either *CCDC88C-FLT3*, a *FLT3* ITD mutant, or an empty vector. Ba/F3 cells transduced with a *FLT3* fusion construct showed IL3-independent growth at a comparable rate to cells expressing a *FLT3* ITD mutant (Fig. 2a). Furthermore, in response to sorafenib drug exposure, *CCDC88C-FLT3*-transduced cells were more sensitive compared with mock cells grown in the presence of IL3 (Fig. 2b). These

data suggest that this *CCDC88C-FLT3* fusion drives cytokine-independent growth and is sensitive to inhibition by sorafenib in vitro. Of note, the fusion junction in *FLT3* in this patient occurs in exon 14, the same site where ITDs occur in AML [9]. *FLT3* ITD-driven AML is associated with resistance to cytotoxic chemotherapy and may explain our patients' lack of response to AML-like chemotherapy [10].

Following sorafenib monotherapy treatment for 2 weeks, the patient's WBC decreased to a normal range. A repeat bone marrow was performed and the patient was noted to be in a cytogenetic remission. Given the novelty of this *CCDC88C-FLT3* fusion, no commercial assays were available to identify fusion transcripts below the sensitivity of cytogenetics. We therefore designed a droplet digital PCR assay to detect levels of the *FLT3* fusion and *FLT3* wild-type transcripts using banked patient samples (Supplemental Fig. 1). Fusion transcript levels in peripheral blood dropped following sorafenib monotherapy and continued to decrease on a combination of azacitidine and sorafenib. This clinical response rendered the patient eligible for HSCT 10 weeks after initiating sorafenib and he received a haploidentical transplant from his father using an alpha/beta T-cell-depleted approach following conditioning with busulfan, cyclophosphamide, thiotepa, and rabbit anti-thymocyte globulin (Fig. 1b). Fusion transcripts reached undetectable levels following HSCT (Fig. 2c) by day +30 and the patient remains on sorafenib monotherapy post-transplant with fusion transcripts remaining undetectable > 300 days after transplant.

There are now several reports of fusions in JMML patients lacking Ras mutations. Interestingly, there is an additional case of a fusion involving t(13;14)(q12;q32) in a pediatric patient with a myeloproliferative neoplasm with hepatosplenomegaly and an elevated monocyte count [11]. Although the fusion genes were not identified in that case, we hypothesize this could also have reflected a *CCDC88C-FLT3* fusion. Recently, several *ALK* and *ROS1* fusions have been reported in JMML patients lacking canonical Ras mutations but did also have monosomy 7 similar to our patient [6]. Fusions involving *FIP1L1-RARA*, *HCMOGT-1-PDGFRB*, *NDEL1-PDGFRB*, and *NUP98-HOXA11* have all been previously reported in JMML patients, several of which would be suspected to respond to targeted therapies, including tyrosine kinase inhibitors [12–15].

In this study, we report and characterize a novel *CCDC88C-FLT3* fusion in a child with JMML that was refractory to cytotoxic chemotherapy. We demonstrated that this *FLT3* fusion transforms Ba/F3 cells and is sensitive to *FLT3* inhibition with sorafenib in vitro. We administered sorafenib, enabling the patient to achieve a molecular remission and proceed safely to HSCT. We also developed a custom ddRT-PCR assay to follow *FLT3* fusion transcript levels throughout his treatment course. In summary, all JMML patients who lack canonical Ras pathway mutations should have RNA-Seq performed to identify potentially targetable fusions including *FLT3*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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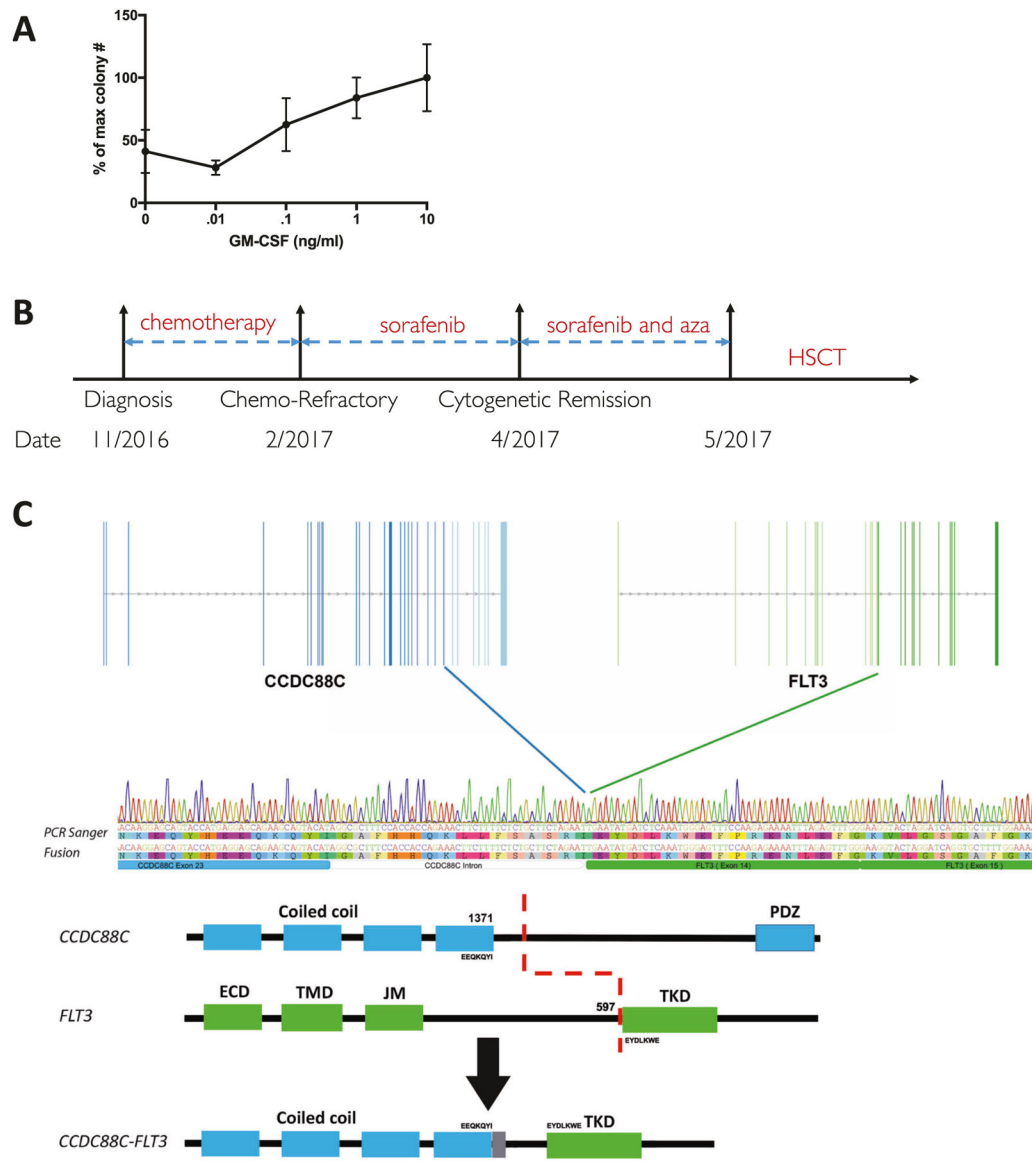
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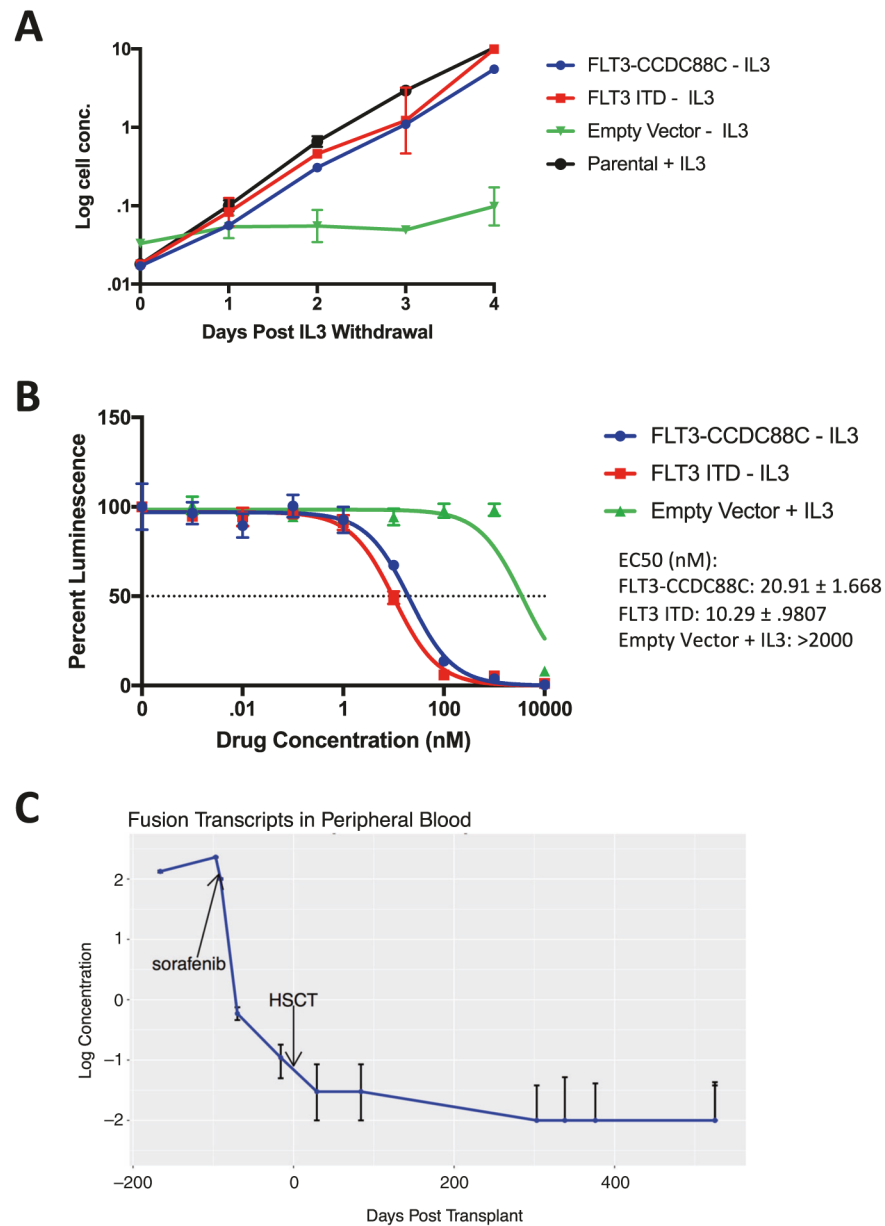


Fig. 2. FLT3 fusion causes cytokine-independent growth and is sensitive to FLT3 inhibition. **a** Growth rates of Ba/F3 cells transduced with *CCDC88C-FLT3*, FLT3 ITD, or mock vector after mouse interleukin-3 (mIL3) withdrawal (mean \pm SEM is displayed, $n = 3$, representative of $N = 3$ independent experiments). **b** Drug sensitivity of Ba/F3 cells to a 48 h sorafenib exposure. EC50 \pm SEM values are reported as the drug concentration that achieves a 50% decrease in luminescence by Cell Titer Glo assay (mean \pm SEM is displayed, $n = 3$, representative of $N = 3$ independent experiments). **c** *FLT3* fusion transcript levels in patient peripheral blood over time as assayed by RT-ddPCR (mean \pm SEM is displayed, $n = 3$, $N = 1$)