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RESEARCH REPORT

Acute myeloid leukemia with an *MN1-ETV6* fusion in a young child with Down syndrome

Jaclyn Rosenzweig,[1] Pallavi M. Pillai,[2] Susan Prockop,[1] Ryma Benayed,[3] Lisa Eidenschink Brodersen,[4] Vesna Najfeld,[5] Michael R. Loken,[4] Yanming Zhang,[3] and Neerav Shukla[1]

[1]Department of Pediatrics, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA; [2]Department of Pediatrics, Division of Pediatric Hematology-Oncology, Mount Sinai Kravis Children's Hospital, Icahn School of Medicine at Mount Sinai, New York, New York 10029, USA; [3]Department of Pathology, Memorial Sloan Kettering Cancer Center, New York, New York 10065, USA; [4]Hematologics, Inc., Seattle, Washington 98121, USA; [5]Department of Medicine and Pathology, Tumor Cytogenomics, Icahn School of Medicine at Mount Sinai Hospital, New York, New York 10029, USA

Abstract Myeloid leukemia of Down syndrome (ML-DS) in young children is associated with distinct clinical and biological features and is typically initiated with oncogenic mutations in the X-linked megakaryocytic transcription factor *GATA1*. Here we present a 3-yr-old child with DS diagnosed with acute myeloid leukemia (AML), which lacks typical immunophenotypic and molecular characteristics of ML-DS, including *GATA1* mutations. The leukemic blasts were found to have an *MN1-ETV6* gene fusion, a high-risk oncofusion not previously described in DS patients. This report highlights the importance of immunophenotypic, cytogenetic, and molecular characterization of ML-DS for identification of rare cases with unique features that may benefit from treatment protocols that are more intensive than those developed for patients with typical *GATA1* mutant ML-DS.

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Corresponding author:
shuklan@mskcc.org

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INTRODUCTION

Children with Down syndrome (DS) due to constitutional trisomy 21 have a 10–20-fold increased relative risk for the development of acute leukemia. Specifically, there is an increased risk of acute megakaryocytic myeloid leukemia (AMKL) (Mezei et al. 2014) The pathogenesis of myeloid leukemia of Down syndrome (ML-DS) in young children is typically initiated with a mutation in the X-linked megakaryocytic transcription factor *GATA1* (Mundschau et al. 2003). Approximately 10%–15% of newborns with DS are diagnosed with transient abnormal myelopoiesis (TAM), a clonal neonatal preleukemic syndrome that is characterized by circulating blasts with acquired mutations in *GATA1*. Frequently, TAM spontaneously resolves, but 10%–20% of newborns with TAM will progress to develop ML-DS through the acquisition of secondary oncogenic mutations. Patients who develop ML-DS are typically diagnosed before the age of 4 yr with half of all patients diagnosed at 1 yr of age (Bhatnagar et al. 2016). ML-DS is characterized by hypersensitivity to chemotherapeutic agents such as cytarabine, allowing for reduced intensity treatment regimens and

leading to significantly better outcomes as compared to non-DS pediatric patients with AML (Sorrell et al. 2012; Taub et al. 2017; Uffmann et al. 2017)

Herein, we describe an unusual case of AML in a 3-yr-old child with DS, characterized by lack of typical immunophenotypic, cytogenetic, and molecular characteristics of ML-DS, including *GATA1* mutations, and by the presence of an oncogenic *MN1-ETV6* gene fusion, who remains in remission with full engraftment for 32 mo following allogeneic stem cell transplantation for refractory disease.

RESULTS

Clinical Presentation

A 3-yr-old male child with trisomy 21 presented with ~5 mo of intermittent fevers with increasing frequency. A complete blood count (CBC) revealed marked leukocytosis with peripheral blasts (white blood cell count, 100.4×10^9/L; blasts, 18×10^9/L), anemia (hemoglobin, 10.9 g/dL), and thrombocytopenia (platelets, 45×10^9/L). Bone marrow evaluation revealed a diagnosis of AML, with expression of CD45 (reduced), HLA-DR (heterogeneous), CD38 (reduced), CD13 (heterogeneous), CD33, CD117 (heterogeneous), dim CD7, and CD123. The abnormal cells lacked expression of CD11b, CD56, CD64, CD36, and the megakaryocytic antigens CD41, CD42b, and CD61. Karyotype analysis showed all 20 metaphase cells with trisomy 21, consistent with the known diagnosis of DS. Furthermore, a three-way translocation t(12;21;22) that results in an *ETV6* translocation and gain of Chromosome 8 were observed, along with a deletion of the long arm of Chromosome 9 in a subclonal population. Fluorescence in situ hybridization (FISH) analysis confirmed the *ETV6* translocation (Fig. 1A,B; Supplemental Fig. 1).

Given the young age of the patient, he was enrolled onto the Children's Oncology Group (COG) protocol AAML1531 for the treatment of ML-DS and started low-intensity induction therapy with thioguanine, cytarabine, and daunorubicin (TAD). On day 15 of induction, a CBC revealed 12% circulating blasts. Bone marrow aspiration confirmed refractory disease with 90% blasts displaying an identical immunophenotype to that at diagnosis. The patient was removed from the study and underwent reinduction with high-dose cytarabine and mitoxantrone, after which he still had persistent disease. During this time, diagnostic targeted RNA profiling (MSK-Fusion) revealed an in-frame fusion between exon 1 of the *MN1* gene on Chromosome 22 and exon 3 of *ETV6* on Chromosome 12 (Fig. 1C; Table 1). Targeted DNA profiling (MSK-IMPACT Heme) did not identify mutations in *GATA1* or any other cancer-related gene. Given the refractory course to initial induction and the poor prognosis of *ETV6* fusions in childhood AML, the patient received further intensive therapy with high-dose cytarabine (Capizzi II schedule) followed by hematopoietic stem cell transplantation (HSCT) from an unrelated 10/10 human leukocyte antigen (HLA)-matched donor (Lugthart et al. 2010; Creutzig et al. 2012). He remains in remission with full donor chimerism 32 mo post-transplant.

Table 1. Variant table

Gene 1 (transcript ID)	Exon of gene 1	Gene 2 (transcript ID)	Exon of gene 2	Fusion junction sequence	Frameshift class
MN1 (NM_002430)	1	*ETV6* (NM_001987)	3	GAACCCCAACAGCAAAGAAGGC *TTACAGCCAATTTACTGG*	In-frame

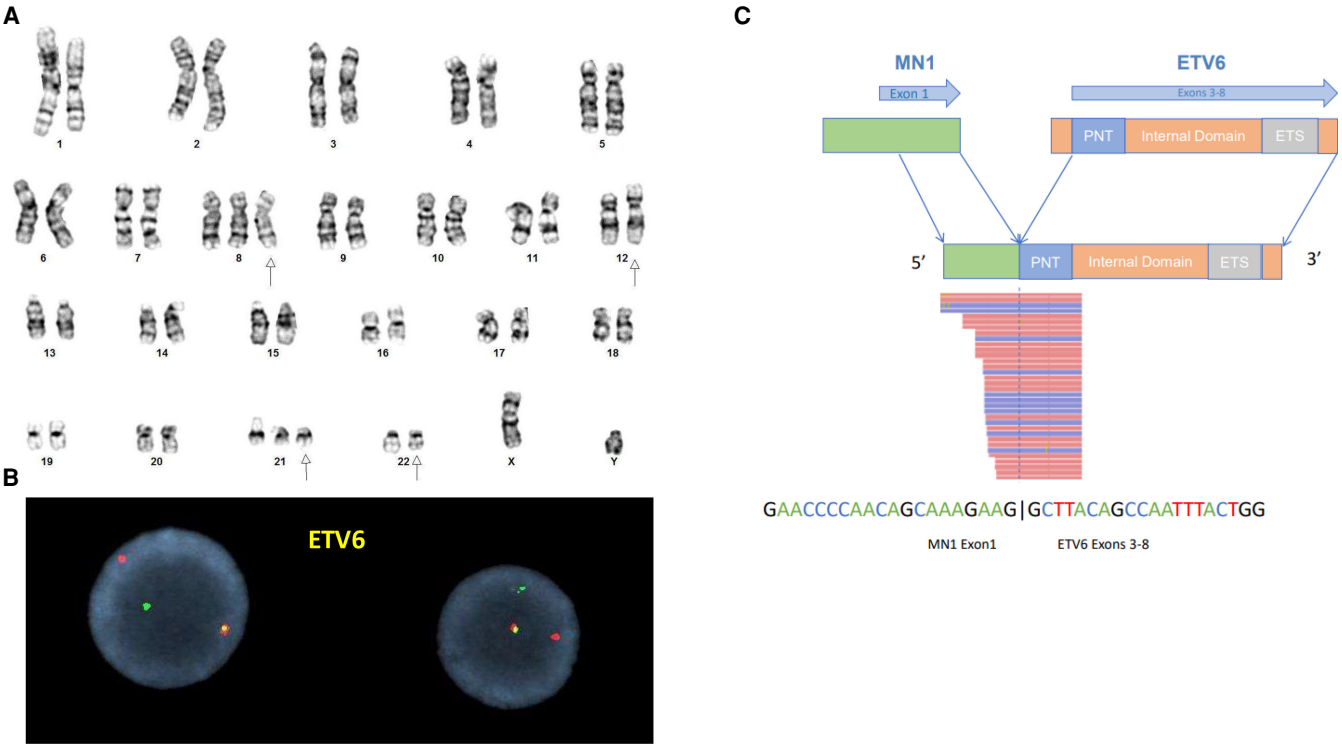

Figure 1. Cytogenetic and molecular evaluation of the patient's leukemia cells at diagnosis. (*A*) Chromosome analysis identifies a clone with constitutional trisomy 21 and somatic gain of Chromosome 8 and t(12;21;22) (arrows). (*B*) Fluorescence in situ hybridization (FISH) analysis using ETV6 break-apart probes shows a split signal pattern of the 5′ and 3′ probes, confirming ETV6 translocation. (*C*) *MN1-ETV6* fusion schematic as identified by RNA sequencing. (PNT) Pointed domain, (ETS) ETS DNA binding domain.

DISCUSSION

To our knowledge, this is the first report of *MN1-ETV6* fusion–driven AML in a pediatric patient with DS. *ETV6* translocations represent a rare subset of pediatric AML. The majority of described cases involve the gene fusion *MNX1-ETV6,* which has been described in up to 30% of AML in patients less than 2 yr of age and is associated with a poor prognosis (Espersen et al. 2018).

ETV6 is a member of the ETS family of transcription factors and contains the pointed protein interaction (PNT) domain and an ETS DNA binding domain (Buijs et al. 2000). *ETV6* is a frequent partner gene of oncogenic fusions across both hematologic and solid tumors (De Braekeleer et al. 2012; Biswas et al. 2020). *MN1* functions as a transcriptional coactivator and has also been found to be overexpressed in subsets of malignancies. Genomic studies have identified *MN1* as a recurrent partner gene in oncogenic fusions in rare pediatric central nervous system tumors (Clarke et al. 2020). Recent studies have demonstrated similarities of the *MN1-ETV6* fusion to *EWS-FLI1* and *PAX3-FOXO3* in terms of structure and oncogenic activity through transcriptional dysregulation (Buijs et al. 2000). *MN1-ETV6* also leads to *MN1* overexpression, resulting in a phenotype similar to *KMT2A* rearranged leukemia with high levels of *HOXA9* and *MEIS1* expression (Wang et al. 2020; Libbrecht et al. 2021). And akin to *KMT2A*-rearranged leukemia, *MN1*-driven leukemias are regulated by the protein menin, leading to a potential therapeutic vulnerability to small-molecule menin inhibitors (Libbrecht et al. 2021).

MN1-ETV6 fusion–driven AML, cytogenetically characterized by t(12;22) translocations, is extremely rare, with less than 50 cases reported in the literature (Wang et al. 2020). In this case, the *MN1-ETV6* fusion transcript resulted from t(12;21;22) (p13;q22;q12), a variant translocation which has not been previously described. Because of similar banding patterns at 12p and 22q, this translocation is subtle and could be easily overlooked in chromosome analysis; FISH analysis using *ETV6* break-apart probes can assist in characterizing the translocation as demonstrated in this case. Shao et al. described 12 AML or MDS patients with t(12;22)(p13;q12) translocation, nine of whom had *MN1-ETV6* identified by FISH or molecular testing (Shao et al. 2018). The only child in the cohort was a 15-yr-old diagnosed with MDS. Outcomes for this group of patients were poor, as 6/7 patients with follow-up data died within 1 yr of diagnosis. Wang et al. described nine patients with t(12;22), including one patient with a complex rearrangement of t(12;17;22) (Wang et al. 2020). Two of the patients were children, including a 4-yr-old diagnosed with AML and an 11-yr-old with mixed phenotype acute leukemia. Six of the nine patients underwent allogeneic transplant, with five of six transplanted patients alive at last follow-up. Trisomy 8 was identified as a recurrent additional abnormality in both reports, as was identified in our patient. No DS patients were reported in either study or any other published case reports.

The development of ML-DS in children younger than 4 yr of age is directly linked to constitutional trisomy 21 and subsequent acquisition of somatic *GATA1* mutations in a well-characterized pathogenic process (Hitzler and Zipursky 2005; Labuhn et al. 2019). Myeloid leukemia in children older than 4 yr of age frequently lack *GATA1* mutations and instead harbor molecular features characteristic of non-DS-associated AML, suggesting a lack of association of the myeloid disease to their underlying trisomy 21 (Hasle et al. 2008). Interestingly, despite being 3 yr of age at diagnosis, our patient's AML did not have evidence of an acquired *GATA1* mutation as assessed by MSK-IMPACT Heme, a 400-gene hybridization capture-based next-generation sequencing assay that tiles all exons of *GATA1* (Ptashkin et al. 2019). Instead, the leukemic cells were characterized by an *MN1-ETV6* fusion along with trisomy 8.

Additionally, our patient was found to have an immunophenotype that was inconsistent with that typical of *GATA1*-associated ML-DS. Typically, blasts from ML-DS are HLA-DR-negative, CD11b-positive, and bright CD117, with bright CD7 positivity and CD36 without CD64 as well as expression of platelet antigens CD41, CD42b, and CD61 (Wilson et al. 2016). However, the blasts from our patient were HLA-DR-positive and CD7-dim with reduced CD45 on the CD34-positive cells, similar to what is seen in AML transformation of MDS in adults (Fig. 2).

Finally, our patient had a highly aggressive disease course with evidence of primary refractory disease 2 wk into induction therapy. Children with ML-DS have superior outcomes and typically respond favorably to chemotherapy as a result of increased sensitivity of ML-DS blasts to both cytarabine and daunorubicin (Taub et al. 2017). Both Sorrell et al. (2012) and Taub et al. (2017) showed that the incidence of refractory disease in patients with ML-DS is low as only 6.4% of patients developed progressive disease in COG trial A2971, and only ~10% of patients experienced treatment failures in COG AAML0431. In contrast to typical *GATA1*-associated ML-DS, AML characterized by *ETV6* fusions is associated with poor outcomes, leading to categorization as a high-risk subtype in ongoing de novo AML trials (Conneely and Stevens 2021). Our patient received intensive reinduction chemotherapy followed by allogeneic HSCT and remains in remission 32 mo post-HSCT.

This case highlights the utility of comprehensive immunophenotypic, cytogenetic, and molecular characterization of ML-DS to identify rare younger patients with DS who develop AML without features of typical *GATA1*-related ML-DS. These patients would likely benefit from intensive treatment protocols designed for sporadic childhood AML. Current reduced-

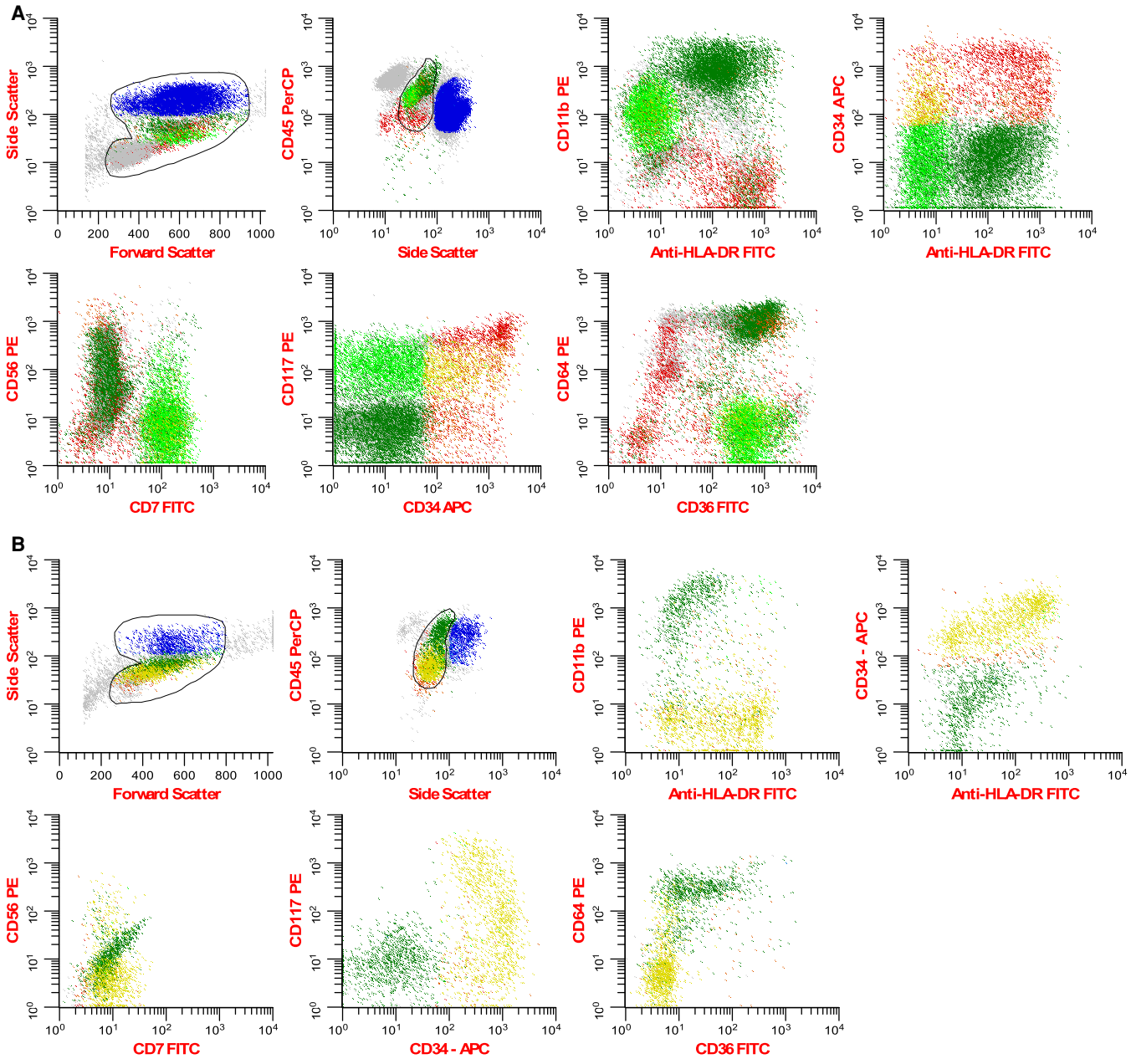

Figure 2. Immunophenotypic comparison between typical *GATA1* mutant myeloid leukemia of Down syndrome (ML-DS) and this patient with *MN1-ETV6* AML. The gating used to identify the abnormal progenitor cells combining forward and side scatter in combination with CD45 is demonstrated in *A* and *B*. (*A*) The recurrent phenotype of *GATA1* mutant ML-DS (bright green) is HLA-DR-negative, CD11b-positive, CD7-bright, CD117-positive, and CD36 without expression of CD64. Expression of other antigens is variable including CD34, CD38, CD13, CD33, and the platelet antigens CD41, CD42b, and CD61. (*B*) The phenotype of the *MN1-ETV6* AML (yellow) was characterized by heterogeneous expression of HLA-DR, dim CD7, and heterogeneous CD117 without expression of CD36. The abnormal cells in this case expressed CD34.

intensity protocols designed for ML-DS commonly use age <4 yr as an eligibility criterion as the vast majority of these patients have canonical *GATA1*-driven AML. Our case highlights that consideration should be given to include immunophenotypic and/or molecular characterization, for identification of driver mutations and oncogenic fusions, as eligibility criteria for proper allocation of children with DS and AML onto clinical trials.

METHODS

The presence of the translocation involving Chromosomes 12, 21, and 22 was identified by standard karyotype analysis and confirmed by a break-apart FISH probe (Fig. 1A,B; Supplemental Fig. 1). Targeted RNA sequencing using a customized Archer FusionPlex panel identified the *MN1-ETV6* transcript involving exon 1 (NM_002430) and exon 3 (NM_001987) of *MN1* and *ETV6*, respectively (Fig. 1C). A detailed description of the Anchored Multiplex Technology is available elsewhere (Zheng et al. 2014). Briefly, Fusion unidirectional Gene Specific Primers (GSPs) have been designed to target specific exons in 132 genes known to be involved in chromosomal rearrangements based on current literature. Complementary DNA (cDNA) undergoes end repair, dA tailing, and ligation with half-functional Illumina molecular barcode adapters (MBCs). Cleaned ligated fragments are subject to two consecutive rounds of polymerase chain reaction (PCR) amplifications using two sets of gene-specific primers (GSP1 pool used in PCR1 and a nested GSP2 pool designed 3′ downstream from GSP1 used in PCR2) and universal primers complementary to the Illumina adapters. This allows for the enrichment of fusion transcripts with the knowledge of only one of the gene partners. At the end of the two PCR steps, the final targeted amplicons are ready for 2 × 150-bp sequencing on an Illumina MiSeq sequencer. At the end of MiSeq sequencing, fastq files are automatically generated using the MiSeq reporter software (Version 2.6.2.3) and analyzed using the Archer analysis software (Version 5.0.4).

ADDITIONAL INFORMATION

Data Deposition and Access

The variant described in this manuscript was deposited in ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) and under the accession number SCV002107492.

Ethics Statement

Informed and signed consent was obtained for the research performed and publication of the results. The patient was enrolled in the Memorial Sloan Kettering Cancer Center (MSKCC) targeted gene sequencing research study (Genomic profiling in cancer patients; NCT01775072) with approval from the MSKCC Institutional Review Board under protocol IRB# 12-245.

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Competing Interest Statement

The authors have declared no competing interest.

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Author Contributions

J.R., P.M.P., S.P., and N.S. conceived the study. L.E.B., M.R.L., R.B., V.N., and Y.Z. provided figures and associated legends. All authors reviewed and approved the final version of the manuscript.

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