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TO THE EDITOR:

Identification of novel *NUP98* fusion partners and comutations in acute myeloid leukemia: an adult cohort study

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Translocations involving the nucleoporin 98 (*NUP98*) and >30 fusion partner genes are well characterized for their involvement in hematological diseases including myelodysplastic syndromes and acute myeloid leukemia (AML).[1] Fusion partners are categorized as homeobox (HOX) class I or class II homeobox genes or nonhomeobox.[1] Class I HOX fusions involves ~10 genes, including *HOXA9*,[2] whereas HOX class II fusion partners are less frequent and includes *HHEX*.[3] Nonhomeobox partner genes, on the contrary, associate with epigenetic regulation and includes *NSD1*, *KDM5A*, and *KMT2A*.[4-6] Expression of fusion transcripts results in oncoproteins with functionality derived from joining of genes. This includes phenylalanine-glycine repeats retained from NUP98 enabling liquid-liquid phase separation and biomolecular condensation of oncoproteins to nuclear puncta combined with functional domains from the partner, including homeodomain, su(var)3-9, enhancer-of-zeste and trithorax, and plant homeodomain fingers.[7] The resultant oncoproteins may directly invoke epigenetic dysregulation or recruit additional factors, promoting leukemogenesis, through activation of distal *HOX* genes and *Meis1*.[8]

Although molecular mechanisms behind *NUP98* translocations are advancing, there is a gap in the understanding of their clinical significance in adult AML cohorts, with literature focusing on pediatric AML.[9,10] This has been reflected with *NUP98* translocations only recently incorporated into the World Health Organization and International Consensus Classification diagnostic classifications; enabling diagnosis of AML, based on detection of <20% blasts (>10% if using International Consensus Classification) and clinical presentation.[11,12] Consequently, existing estimations regarding adult *NUP98* malignancies may underrepresent their true frequency. To address this gap, we performed the most complete screening to date on *NUP98* translocations in an adult AML cohort, combining fluorescent in situ hybridization (FISH) and next-generation sequencing (NGS).

Our sex-matched cohort of 291 adults, with a median age of 63.5 years, included 161 males and 130 females ($P = .079$, Fischer exact test), consisted of 199 retrospective adult patients with AML diagnosed between 2016 and 2021 and 92 adult patients with AML diagnosed prospectively in 2022. Eight cases of *NUP98* translocation (2.8%) were detected (Table 1), significantly affecting younger adults (median, 45 years; range, 31-67), compared with that of patients without *NUP98* translocations ($P = .0002909$, unpaired *t* test; Figure 1A). A clear male sex bias in patients positive for *NUP98* translocation was also observed (7 males; 1 female) (Table 1). Four cases were de novo AML (2 acute myelomonocytic, 1 AML with minimal differentiation, and 1 AML with maturation), with 4 cases of myelodysplasia-related changes AML (Table 1).

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Data are available on reasonable request from the corresponding author, María Berdasco (mberdasco@carrerasresearch.org).

The full-text version of this article contains a data supplement.

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Table 1. Clinical, cytogenetic, and NGS data from patients with AML with *NUP98* translocation

ID	Diagnosis (WHO 2016)	Age	Sex	Year	WBC, $\times 10^9$/L	HB, g/L	PLT, $\times 10^9$/L)	Blast % BM	Treatment	CR	Status	Karyotype	FISH	Breakpoint and fusion partner (*NUP98*::X)
1	AML with myelodysplasia-related changes	43	M	2016	15.2	69	50	18*	CETLAM AML-12	Yes†	Alive	46,XY[30]	1R 1G 1Fus	12::6 *NSD1*
2	Acute myelomonocityc leukemia	65	M	2017	32.4	55	55	35	Quantumfirst TRIAL	Yes	Died in CR	46,XY[20]	1R 1Fus	12::2 *EMX1*
3	AML with myelodysplasia-related changes	39	M	2020	285	71	29	86	CETLAM AML-12	No	Died in progression	45,XY,add(1)(p36),del(1)(p15),?inv(14)(q11q32),der(17)t(17;22)(p11.2;p11.2),?inv(18)(q21.3q23),-22[20]	1R 1G 1Fus	12::6 *NSD1*
4	AML with myelodysplasia-related changes	49	M	2020	40.75	72	115	55	CETLAM AML-12	Yes‡	Died in progression	46,XY[20]	1R 1G 1Fus	12::6 *NSD1*
5	Acute myelomonocityc leukemia	52	F	2022	327	43	46	81	HOVON 156 TRIAL	No	Died in progression	46,XX[25]	1R 2Fus	12::6 *NSD1*
6	AML with maturation	31	M	2022	7.93	86	160	27	CETLAM AML-12	No	Died during induction (septicemia)	46,XY,inv(11)(p15q23)]	1R 1G 1Fus	14::2 *KMT2A*
7	AML with minimal differentiation	33	M	2022	1.3	95	285	83	VENAZA	Yes	Alive	47,XY,inv(11)(q13.5q25),del(12)(p13),+21c [2] / 46,X,-Y,inv(11)(q13.5q25),del(12)(p13),+21c [18]	1Fus 1R	12::26 *KDM5A*
8	AML with myelodysplasia-related changes	48	M	2022	238	73	47	70	KB-LANRA-1001 Trial	Yes	Alive (relapsed after allo-sct)	46,XY[30]	1R 1G 1Fus	12::6 *NSD1*

add, additional material of unknown origin; CR, complete remission; del, deletion; *EMX1*, Empty Spiracles Homeobox 1; F, female; Fus, fusion; G, green; HB, hemoglobin; inv, inversion; *KDM5A*, lysine demethylase 5A; *KMT2A*, lysine methyltransferase 2A; M, male; *NSD1*, nuclear receptor binding SET domain protein 1; PLT, platelet count; R, red; VENAZA, venetoclax plus azacitidine protocol; WBC, whole-blood cells.
*Peripheral blood 29%.
†Relapsed before allogeneic stem cell transplant that was rescued.
‡Relapsed before allogeneic stem cell transplant that could not be rescued.

Concordant with previous adult studies, most *NUP98* translocations were cytogenetically normal (n = 5), with 3 exceptions displaying cytogenetic aberrations to chromosome 11 (Table 1).[13-17] This included an interstitial deletion p15, a pericentric inversion p15-q23, and a paracentric inversion q13.5q25. FISH confirmed *NUP98* translocations in all patients (supplemental Figure 1A-G), with a targeted NGS panel detecting fusion breakpoints, comutations, and identification of fusion partners.[18] Intronic breakpoints between exons 12 and 13 of *NUP98* were the most frequent (87.5%), with 1 breakpoint between exons 14 and 15 (Table 1; supplemental Figure 1H). All patients harbored comutations, in total involving 9 genes (Figure 1B). Comutations in W*T1* and *FLT3*-ITD (internal tandem duplications) were the most frequent, occurring in 5 of 8 patients (62.5%), and were enriched alongside *NUP98*::*NSD1* translocation. Finally, comutations were detected in *RAD21*, *JAK1*, and *DNMT3A,* all previously unreported alongside *NUP98* translocations in AML. Overall, 4 fusion partners were detected (Figure 1B), concordantly agreeing with literature that *NUP98*::*NSD1* rearrangements are the most frequent in adult AML (n = 5).[14] Additionally, 3 fusions observed in single cases of clinical importance were detected, including an undocumented fusion partner, Empty Spiracles Homeobox 1 (*EMX1*), named *NUP98*::*EMX1*; a rare report of *NUP98*::*KMT2A*; and the first adult case of *NUP98*::*KDM5A* (Table 1).

The first fusion observed in a single case involved rearrangement of *NUP98* with the antennapedia class homeobox family gene *EMX1*, detected from a cytogenetically normal patient sample of a 65-year-old male diagnosed with AML (supplemental Figure 2A). FISH detected an atypical split signal, with deletion of a distal 3' green-end signal (supplemental Figure 2B). NGS revealed the fusion between exon 12 of *NUP98* and exon 2 of the Class II HOX gene and comutations in *U2AF1* and *FLT3*. Reverse transcription polymerase chain reaction using complementary DNA produced from patient bone marrow and a forward primer in exon 12 of *NUP98* and a reverse primer in exon 2 of *EMX1* confirmed expression of the novel fusion vs a *NUP98*::*NSD1* control (supplemental Figure 2C). A full-length *NUP98*::*EMX1* complementary DNA transcript was generated using a forward primer at the start of exon 1 of *NUP98* and a reverse primer at the end of exon 3 of *EMX1*. Subsequent cloning into the pLVX-Puro vector, followed by Sanger sequencing confirmed an in-frame fusion transcript fusion between *NUP98* exon 12 with *EMX1* exon 2 (supplemental Figure 2D). This included retention of phenylalanine-glycine repeats from *NUP98* (amino acids 80-152 and 253-332) and exons 2 and 3 of *EMX1*, retaining the homeobox domain (amino acids 192-251) and disordered region (amino acids 249-290; supplemental Figure 2E). The second fusion observed in a single case included a 31-year-old male diagnosed with AML with maturation, with pericentric inversion of chromosome 11 and a karyotype of 46,XY,inv(11)(p15q23) (supplemental Figure 3A). NGS revealed *NUP98* translocation with *KMT2A*, with a metaphase FISH confirmation of rearrangement (supplemental Figure 1A). In addition, NGS detected the well-known missense hot spot mutation, c.2645G>A in *DNMT3A*, representing a very rare genetic event in AML, with *DNMT3A* comutated alongside *NUP98* rearrangement AML. Critically, this rare case, to our knowledge, represents the third reported case in AML of *NUP98*::*KMT2A* and, consistent with previous cases, involves the same pericentric inversion of chromosome 11 inv(11)(p15q23).[6]

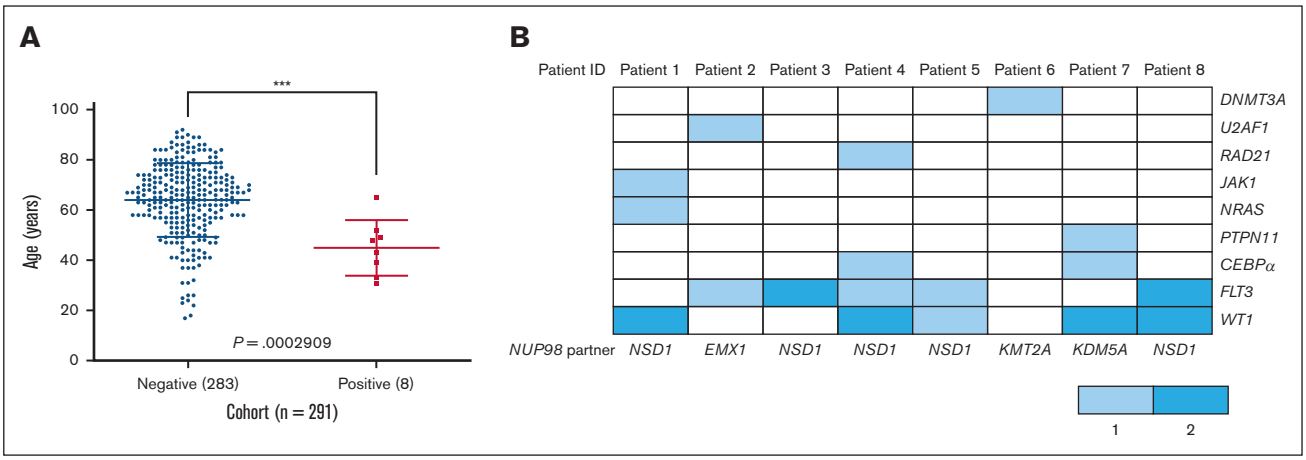

Figure 1. Clinical and genetic characteristics of adult patients with AML with NUP98 translocations. (A) Ages of adult patients with AML without *NUP98* rearrangement (n = 283) compared with those with *NUP98* rearrangement (n = 8). (B) Comutations detected in adult patients with AML with *NUP98* rearrangement through targeted NGS. The scheme shows the number of mutations per patient, with the corresponding *NUP98* fusion partner indicated along the lower x-axis, the patient ID along the upper x-axis, and the comutated gene along the y-axis. The number of mutations per gene are determined by color; 1 detected mutation is represented by light blue and 2 mutations in the same gene by dark blue.

Finally, the third fusion observed in a single case includes a 33-year-old male with Down syndrome diagnosed with AML with minimal differentiation and a karyotype of 47,XY,inv(11)(q13.5q25),del(12)(p13),+21c[2]/46,X,-Y,inv(11)(q13.5q25),del(12)(p13),+21c[18] (supplemental Figure 3B). FISH revealed a single joint fusion and deletion of a distal 3'-green probe (supplemental Figure 1F), with NGS detecting translocation with *KDM5A* and mutations in *PTPN11* and *CEBPA* and 2 mutations in *WT1*. Despite the characterization of *NUP98*::*KDM5A* in pediatric AML, we believe this case represents, to our knowledge, the first reported case of *NUP98*::*KDM5A* translocation involvement in adult AML, doubling as the first reported *NUP98* translocation AML case with Down syndrome.

Herein, we present results from analyzing a large series of nearly 300 adult patients with AML using FISH and NGS. We report *NUP98* translocations are more frequent in adult AML than the summarized adult studies estimate (supplemental Table 1), reporting an occurrence of 2.8%.[13-17] Our results also challenge previously assumed equal sex distributions of *NUP98* translocations in adult AML, reporting a notable male sex bias (7:1).[14,16,17] Three clinically interesting cases were also detected, including the novel fusion *NUP98*::*EMX1*. Believed to be involved in the determination of cellular identity during corticogenesis, *EMX1* and its paralogue *EMX2* may also act as tumor suppressors in solid tumors; however, no reports currently link *EMX1* to hematological disease.[19,20] Finally, we provide, to our knowledge, the first evidence that *NUP98*::*KDM5A* is not exclusive to pediatric or non–Down syndrome AML and report a rare case of *NUP98*::*KMT2A* in AML.[6] Alongside this, extremely rare comutations including *DNMT3A*, *JAK1*, and *RAD21* were detected, expanding the small mutagenome associated with *NUP98* rearrangements. Crucially, through an improved understanding of comutations, this could inform new treatment strategies, including JAK inhibitors or epidrug trials.[21] Furthermore, due to the technical success highlighted by this study that NGS and FISH possess in detecting *NUP98* translocations, we recommend their use in clinical evaluation, particularly on cytogenetically normal patients with AML. This may prove important in guiding more appropriate treatment strategies, such as myeloablative therapy, which may be more tolerated and benefit the typically younger patients affected by *NUP98* translocation AML. To conclude, our findings offer valuable clinical insights into AML, opening potential avenues for therapeutics and research, while providing a clinical reference for *NUP98* translocations in adult AML.

All patient samples included in the study were obtained from the unit of collection of samples of Josep Carreras Leukaemia Research Institute after their respective institutional review board and ethical approval (ethics committee at Hospital Universitari Germans Trias I Pujol Ref. PI-20-278; date of approval: 25 September 2020).

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data; J.S.H. prepared the tables and figures; J.S.H. and M.B. wrote the manuscript; and all authors have reviewed and agreed to publish the manuscript.

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