DDX3X-MLLT10 fusion in adults with *NOTCH1* positive T-cell acute lymphoblastic leukemia

MLLT10 (also known as *AF10*), at chromosome 10 band p12, is emerging as a promiscuous gene. Six partners have been reported to date: *PICALM(CALM)*/11q14, *MLL*/11q23, *NAP1L1*/12q21, *HNRNPH1*/5q35, *DDX3X*/Xp11.3 and *NUP98*/11p15.[1,2] All fusions retain the MLLT10 octapeptide motif-leucine-zipper (OM-LZ) domain which induces acute myeloid leukemia in mouse models, suggesting it is critical for leukemogenesis. In pediatric T-cell acute lymphoblastic leukemia (T-ALL) *PICALM-MLLT10, HNRNPH1-MLLT10* and *DDX3X-MLLT10* fusions shared a specific gene expression profile signature which included NK-like homeobox overexpression, differentiating it from all other genomic rearrangements in the *HOXA* category.[1]

Focusing on *MLLT10* involvement in adult T-ALL, we used fluorescence *in situ* hybridization (FISH) to investigate 99 patients (31 females, 68 males; age range 14-69 years, median age 34) who were enrolled in 2 consecutive multicenter GIMEMA (Gruppo Italiano Malattie Ematologiche dell'Adulto) studies (protocols 0904 and 0496). All patients provided informed consent for sample collection. Biological analyses were carried out in accordance with the Declaration of Helsinki. Screening studies were approved by the Institutional Review Board of "Sapienza" University, Rome, Italy.

In these 99 patients, the overall incidence of *MLLT10* translocations was approximately 10% (10 of 99 patients). FISH investigated for known *MLLT10* partners,[1,3] finding 7 patients with *PICALM-MLLT10* and 3 with *DDX3X-MLLT10*. A biological sample from a 4th patient was obtained when a parallel study using whole transcriptome sequencing on a Illumina HiSeq2000 (Illumina, San Diego, CA, USA) instrument identified an additional case harboring the *DDX3X-MLLT10* fusion transcript in a different cohort of 20 adult T-ALL patients.[4] Table 1 reports clinical, hematologic and cytogenetic findings in all 4 *DDX3X-MLLT10* positive T-ALL patients. All were males presenting with high white blood cells count. In case 1, T-ALL was arrested at cortical stage as previously described in a child.[1] In the other 3 patients, immunophenotype was incomplete or not available. All achieved hematologic remission but 3 relapsed and died. One patient (n. 1) is alive and well at 80 months post-HLA identical stem cell transplantation (HLA-SCT).

A *DDX3X-MLLT10* double color double fusion FISH assay detected balanced translocations in 3 cases and unbalanced in 1 (Figure 1A-C). Reverse-transcription polymerase chain reaction (Thermoscript RT-PCR System, Invitrogen, Carlsbad, CA, USA) and sequencing (AB3500 Genetic analyzer, Applied Biosystem, Foster City, CA, USA) confirmed in-frame *DDX3X-MLLT10* transcripts in all patients but breakpoints differed from our previously described pediatric case. *DDX3X* exon 6 fused to *MLLT10* exon 6 in Patient 1 and to exon 4 in Patient 4; *DDX3X* exon 3 fused to *MLLT10* exon 17 in Patient 2 and *DDX3X* exon 4 fused to *MLLT10* exon 7 in Patient 3 (Figure 1D). An additional splicing variant joining *DDX3X* intron 6-7 to *MLLT10* intron 5-6 was identified by RNA-seq in Patient 1. A reciprocal *DDX3X-MLLT10* in-frame fusion was detected only in Patients 1 and 2, supporting the hypothesis that *DDX3X-MLLT10* is critical in the pathogenesis of this T-ALL subgroup.[1]

Table 1. Clinical, hematologic and cytogenetic findings in all 4 DDX3X-MLLT10 positive T-ALL patients.

Pts	S	A	WBC (x10^9/L)	Immunophenotype	Karyotype	CI-FISH	SNP analysis	NOTCH1	CNOT3	Therapy	Follow up (months)
1	M	26	88	cortical: CD45$^+$,	46,Y,t(X;10)(p11;p12),	del(5q)/*MAPK9,FLT4*	n.a.	c.4778T>C;	c.242 G>A;	GIMEMA	+80
				cyCD3$^+$, TdT$^+$, sCD3$^+$, CD4$^+$, CD5$^+$, CD$^+$, CD38$^+$, CD1a$^+$, CD34$^-$, CD10$^-$	add(1)(p36),del(9)(p11p24)[10] 46,idem,del(6q15)[1] 46,XY[11]	del(6)(q15)/*CASP8AP2* del(9)(p21)/*CDKN2A/B*		p.L1593P c.7541_7542del CT; p.P2514RfsX3	p.R81H	0904* HLA identical SCT	alive
2	M	38	160	TdT$^+$, CD7$^+$, cyCD3$^+$,	n.a.	°del(9)(p21)/	**LOSS:**	c.4778 T>C; p.L1593P	normal	GIMEMA	relapse
				CD5$^+$, CD4$^+$, CD8$^-$,		*CDKN2A/B*	2p22.2, 9p21.3, 10q26.12-26.3, 21q11.2-22.3 **LOH:** 9p24.3-21.3	c.5116 C>T; p.L1706F c.7171 C>T; p.Q2391X		0904*	+38 dead
3	M	31	700	n.a.	46,XY[20]	normal	**LOSS:**	c.5087C>A; p.A1696D	normal	GIMEMA	relapse
							1p12-21.3 **GAIN:** 6q23.3	c.7021dupT; p.S2341FfsX13		0904*	+10 dead
4	M	16	68	CD45$^+$, TdT$^+$, HLA-DR$^+$,	46,XY[20]	§del(9)(p21)/	LOSS:				
				CD7$^+$, CD2$^+$, sCD3$^+$, CD5$^+$, CD8$^+$, CD19$^-$, CD14$^-$, CD13$^-$, CD33$^-$, CD34$^-$, CD22$^-$, CD20$^-$, CD4$^-$		*CDKN2A*	1q32.1, 7q34, 9p21.3 **LOH:** 9p24.3-21.3	c.7378G>T; p.E2460X	c.1941delC; p.Y648TfsX44	GIMEMA 0496* unrelated SCT	relapse +24 dead

Pts: patients; S: sex; A: age (years); WBC: white blood cells; cy: cytoplasmic; s: surface; n.a.: not available; CI-FISH: combined interphase fluorescence in situ *hybridization investigated: LEF1,del(5)(q35)/TLX3-MAPK9-FLT4,CASP8AP2-GRIK2,IKZF1,CMYC,CDKN2A/B,ABL1,NUP214,PTEN,WT1,ETV6,BCL11B,NF1,PTPN2; °biallelic deletion of 2 fosmids G248P82010F5 (encompassing CDKN2A) and G248P8257D2 (encompassing CDKN2B); §biallelic deletion of G248P82010F5; SNP,single nucleotide polymorphism; HLA: human Leukocyte Antigen; SCT: stem cell transplantation; *Vitale A.* et al.[5]

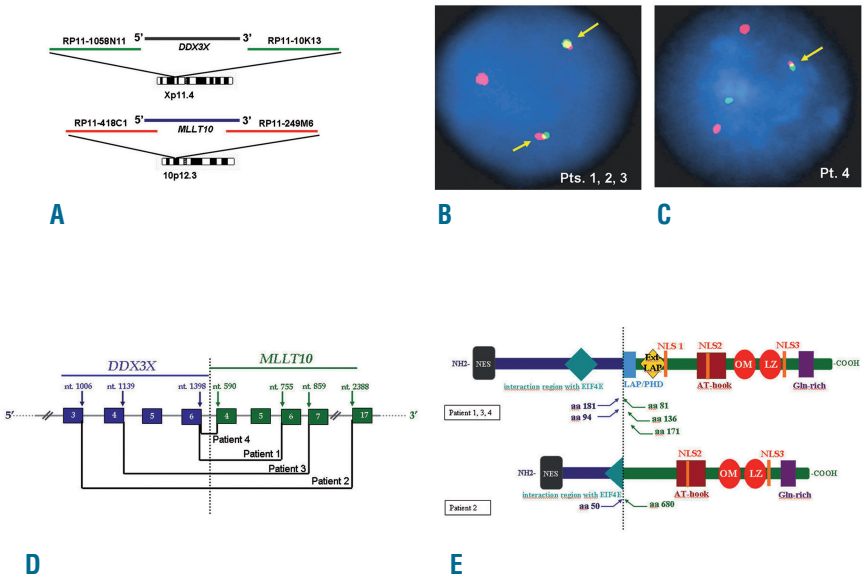

Figure 1. Cytogenetic and molecular characterization of *DDX3X-MLLT10* fusions. (A) Double color double fusion FISH assay for *DDX3X* and *MLLT10*. (B) FISH showed 2 fused signals in Patients 1, 2 and 3 indicating a balanced translocation (arrows). (C) FISH showed one fused signal in patient 4 indicating an unbalanced translocation (arrow). (D) Schematic representation of *DDX3X* and *MLLT10* breakpoints in the 4 *DDX3X-MLLT10* positive cases (arrows). Nucleotide numbers refer to GenBank accession: NM_001356.3 for *DDX3X* and NM_004641.3 for *MLLT10*. (E) Putative fusion protein structure. At N terminal DDX3X retained a NES domain in all. Three patients retained the entire EIF4E interacting motif and 1 only half. At C terminal at least 1 NLS, the AT-hook and the OM-LZ domain were retained in all. Pt: patient; Pts: patients; nt.: nucleotide; aa: amino acid; NES: Nuclear Exporting Signal; NLS: Nuclear Localization Signal; LAP/PHD: Leukemia Associated Protein / Plant Homeo Domain; Ext-LAP: Extended LAP; OM-LZ: Octapeptide Motif-Leucine Zipper; Gln: Glutamine.

The *MLLT10* leukemogenic OM-LZ domain and at least one nuclear localization signal were maintained at the C-terminal in all fusions (Figure 1E). At the N-terminal, DDX3X retained a nuclear export signal (NES) domain, which interacts with CRM1, and an EIF4E interacting motif that is required for *DDX3X* modulation of translation. The contributions of these domains to *DDX3X-MLLT10* leukemogenesis remains to be established. The PICALM CRM1-dependent NES was shown to play a major role in the onset of *PICALM-MLLT10* positive leukemias. Nuclear export of *PICALM-MLLT10* mislocalized a DOT1L H3K79 methyltransferase fraction to outside the nucleus, with loss of H3K79 methylation overall except for critical genes such as *HOXA* which were hypermethylated at lysine 79 and up-regulated.[6] Interestingly, a potent and selective inhibitor of DOT1L is under evaluation to enter human clinical trials as a target therapy for acute leukemias bearing *MLL* translocations.[7]

Finding *DDX3X* in these 4 patients with T-ALL ranked it as the second most frequent *MLLT10* partner. *DDX3X*, an ubiquitously expressed gene, belongs to the adenosine 5'-triphosphate-dependent DEAD box RNA helicases family and has been recurrently involved in solid and hematologic tumors. *DDX3X* is located at Xp11.3 and it is one of the genes that escapes X-inactivation in females.[8] As all patients with *DDX3X-MLLT10* positive T-ALL (the 4 adults in this study and 1 child previously described)[1] were males, no wild-type *DDX3X* allele was retained in the leukemic blasts, suggesting that the complete absence of a normally functional DDX3X protein might contribute to leukemogenesis. *DDX3X* appeared to have oncogenic as well as tumor suppressor functions.[9,10] *DDX3X* somatic mutations have recently been discovered in medulloblastoma,[11,12] chronic lymphocytic leukemia,[13] and Burkitt lymphoma.[14] Recurrent *DDX3X* homozygous deletions were identified in gingivo-buccal oral squamous cell carcinoma.[15]

To identify concurrent molecular hits in our *DDX3X-MLLT10* positive T-ALL, Combined interphase-FISH for recurrent T-ALL associated rearrangements,[3] SNP array and Sanger sequencing for *NOTCH1* and *CNOT3* were performed (Table 1). *NOTCH1* mutation was common to all. *CDKN2A/B* deletions and *CNOT3* mutations appeared to be accompanying recurrent events in 3 and 2 cases, respectively. *CNOT3*, a putative tumor suppressor gene, has been recently reported to be mutated in approximately 8% of adult T-ALL.[16] Other genomic imbalances, as well as copy neutral loss of heterozygosity, were identified in individual cases.

In our study, longitudinal molecular studies were conducted only in Patients 3 and 4 because of lack of biological material in the others. Both patients achieved hematologic remission, but the *DDX3X-MLLT10* fusion persisted after consolidation in Patient 3, and during maintenance in Patient 4 using a nested PCR.

In conclusion, we report for the first time that *DDX3X-MLLT10* occurs in approximately 3% of adult T-ALL and characterizes a subgroup of *NOTCH1* positive leukemias. The *DDX3X-MLLT10* fusion behaved as a primary abnormality and occurred alternatively to rearrangements of other T-cell oncogenes, such as *TAL1, TAL2, LMO1, LMO2, TLX1, TLX3*, and *NKX2-1*. *CDKN2A/B* deletions and *CNOT3* alterations were frequent co-operating hits. *DDX3X-MLLT10* appeared to be a stable and reliable molecular marker for monitoring residual disease. Although its prognostic impact can only be assessed in a much larger cohort of patients, it is worth noting the only long-term survivor underwent HLA-SCT. Diagnosis of *MLLT10* positive leukemias will be helpful to select candidates to target therapy with DOT1L inhibitor.

Lucia Brandimarte,[1] Roberta La Starza,[1*] Valentina Gianfelici,[2*] Gianluca Barba,[1] Valentina Pierini,[1] Danika Di Giacomo,[1] Jan Cools,[3] Loredana Elia,[2]*

Antonella Vitale,[2] Luigiana Luciano,[4] Antonella Bardi,[5] Sabina Chiaretti,[2] Caterina Matteucci,[1] Giorgina Specchia,[6] and Cristina Mecucci[1]

[1]Hematology and Bone Marrow Transplantation Unit, University of Perugia, Italy; [2]Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy; [3]Laboratory for the Molecular Biology of Leukemia, Center for Human Genetics, KU Leuven and Center for the Biology of Disease, VIB, Leuven, Belgium; [4]Hematology, University of Federico II, Naples, Italy; [5]Institute of Hematology, Department of Biomedical Sciences, Azienda Ospedaliero Universitaria Sant'Anna, Ferrara, Italy; and [6]Emergency Department, Section of Hematology, University of Bari, Italy

**LB, RLS and VG contributed equally to this manuscript.*

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Correspondence: cristina.mecucci@unipg.it.
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