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by Lucia Brandimarte, Roberta La Starza, Valentina Gianfelici, Gianluca Barba, Valentina Pierini, Danika Di Giacomo, Jan Cools, Loredana Elia, Antonella Vitale, Luigiana Luciano, Antonella Bardi, Sabina Chiaretti, Caterina Matteucci, Giorgina Specchia, and Cristina Mecucci

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

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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. 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.

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, median 34) who were enrolled in two consecutive multi-centre GIMEMA (Gruppo Italiano Malattie Ematologiche dell’Adulto) studies (protocols 0904 and 0496). All patients provided informed consent for sample collection. Biological analyses were done in accordance with Helsinki Declaration. 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 ~10% (10/99). FISH investigated for known MLLT10 partners, 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 harbouring the DDX3X-MLLT10 fusion transcript in a different cohort of 20 adult T-ALL patients. Table 1 reports clinical, hematological 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. In the other 3 patients
immunophenotype was incomplete or not available. All achieved hematological remission but 3 relapsed and died. One patient (no. 1) is alive and well at 80 months post-HLA identical stem cell transplantation (HLA-SCT).

A **DDX3X-MLLT10** double colour 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) and sequencing (AB3500 Genetic analyzer, Applied Biosystem, Foster City, CA) 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 **MLLT10-DDX3X** 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

The **MLLT10** leukemogenic OM-LZ domain and at least 1 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 upregulated.⁵ Interestingly a potent and selective inhibitor of DOT1L is under
evaluation to enter in human clinical trials as a target therapy for acute leukemias bearing MLL translocations.\(^6\)

Finding \textit{DDX3X} in these four patients with T-ALL ranked it as the second most frequent \textit{MLLT10} partner. \textit{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 hematological tumours. \textit{DDX3X} is located at Xp11.3 and it is one of the genes that escapes X-inactivation in females.\(^7\) As all patients with \textit{DDX3X-MLLT10} positive T-ALL (4 adults herein included and 1 child previously described)\(^1\) were males no wild type \textit{DDX3X} allele was retained in the leukemic blasts suggesting the complete absence of a normally functional \textit{DDX3X} protein might contribute to leukemogenesis. \textit{DDX3X} appeared to have oncogenic as well as tumor suppressor functions.\(^8,9\) \textit{DDX3X} somatic mutations have recently been discovered in medulloblastoma,\(^10,11\) chronic lymphocytic leukemia\(^12\) and Burkitt Lymphoma.\(^13\) Recurrent \textit{DDX3X} homozygous deletions were identified in gingivo-buccal oral squamous cell carcinoma.\(^14\)

To identify concurrent molecular hits in our \textit{DDX3X-MLLT10} positive T-ALL, Combined Interphase-FISH for recurrent T-ALL associated rearrangements,\(^3\) SNP array and Sanger sequencing for \textit{NOTCH1} and \textit{CNOT3} were performed (Table 1). \textit{NOTCH1} mutation was common to all. \textit{CDKN2A/B} deletions and \textit{CNOT3} mutations appeared to be accompanying recurrent events in 3 and 2 cases, respectively. \textit{CNOT3}, a putative tumor suppressor gene has been recently reported to be mutated in about 8% of adult T-ALL.\(^15\) Other genomic imbalances as well as copy neutral loss of heterozygosity were identified in individual cases.

Longitudinal molecular studies were conducted only in patients nos. 3 and 4 because of lack of biological material in the others. Both patients achieved haematological remission but the \textit{DDX3X-MLLT10} fusion persisted after consolidation in case no. 3, and during maintenance in case no. 4 using a nested PCR.
In conclusion we report for the first time that \textit{DDX3X-MLLT10} occurs in about 3% of adult T-ALL and characterizes a subgroup of \textit{NOTCH1} positive leukemias. The \textit{DDX3X-MLLT10} fusion behaved as a primary abnormality and occurred alternatively to rearrangements of other T-cell oncogenes, such as \textit{TAL1}, \textit{TAL2}, \textit{LMO1}, \textit{LMO2}, \textit{TLX1}, \textit{TLX3}, and \textit{NKX2-1}. \textit{CDKN2A/B} deletions and \textit{CNOT3} alterations were frequent co-operating hits. \textit{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 \textit{MLLT10} positive leukemias will be helpful to select candidates to target therapy with DOT1L inhibitor.

\textbf{Authorship and Disclosures}

L.B. designed and performed molecular experiments and drafted the paper; R.L.S. supervised FISH experiments and drafted the paper; V.G. and J.C. performed RNA sequencing experiments; G.B. performed SNPs analysis; V.P. performed karyotype analysis, selected DNA clones and performed FISH experiments; D.D.G., L.E., A.V, L.L., A.B, S.C., Ca. M., G.S. provided clinical, immunophenotypic, cytogenetic and molecular data of patients; C.M. designed the study, supervised all the results and wrote the paper. The authors report no potential conflicts of interest.
References


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

<table>
<thead>
<tr>
<th>Pts</th>
<th>S</th>
<th>A</th>
<th>WBC (x10^9/L)</th>
<th>Immunophenotype</th>
<th>karyotype</th>
<th>CI-FISH</th>
<th>SNP analysis</th>
<th>NOTCH1</th>
<th>CNOT3</th>
<th>Therapy</th>
<th>Follow-up months</th>
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<tr>
<td>1</td>
<td>M</td>
<td>26</td>
<td>88</td>
<td>cortical: CD45+, cyCD3+, TdT+, sCD3+, CD4+, CD5+, CD7+, CD38+, CD1a+, CD34+, CD10-</td>
<td>46,Y,t(X;10)(p11;p12),add(1)(p36),del(9)(p11,1p24)[10]</td>
<td>del(5q)/MAPK9,FLT4 del(6)(q15)/CASP8AP2 del(9)(p21)/CDKN2A/B</td>
<td>n.a.</td>
<td>c.4778T&gt;C; p.L1593P</td>
<td>c.7541_7542del CT; p.P2514RfsX3</td>
<td>GIMEMA 0904* HLA identical SCT</td>
<td>+80 alive</td>
</tr>
</tbody>
</table>

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-GRK2,IKZF1,CMYC,CDKN2A/B,ABL1,NUP214,PTEN,WT1,ETV6,BCL11B,NF1,PTPN2; * biallelic deletion of 2 fosmids G248P82010F5 (encompassing CDKN2A) and G248P82010F5 (encompassing CDKN2B); § biallelic deletion of G248P82010F5; SNP, single nucleotide polymorphism; HLA, Human Leukocyte Antigen; SCT, stem cell transplantation; * Vitale A. et al. Blood. 2006;107(2):473-9.
**Figure Legend**

**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 patient 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.