NAP1L1-MLLT10 is a rare recurrent translocation that is associated with HOXA activation and poor treatment response in T-cell acute lymphoblastic leukaemia

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MLLT10 (previously AF10) was originally described as a fusion partner of the KMT2A (previously MLL) gene in acute myeloid leukaemia (AML) (Beverloo et al, 1995), and was subsequently found to be recurrently rearranged in both AML and T-acute lymphoblastic leukaemia (T-ALL) (Dreyling et al, 1998). PICALM (previously CALM) is the most frequent MLLT10 translocation partner in T-ALL, where it is found in 6-7% of cases (Ben Abdelali et al, 2013). The list of MLLT10 partners in T-ALL now also comprises NAP1L1 (Zhang et al, 2012), HNRNPH1, DDX3X (Brandimarte et al, 2013) and XPO1 (Bond et al, 2014). While DDX3X-MLLT10 has been estimated to occur in about 3% of adult T-ALL cases (Brandimarte et al, 2014), all other fusion transcripts have to date been described in single cases only, and their precise incidence remains to be defined.

The NAP1L1-MLLT10 rearrangement was originally discovered by whole genome sequencing of leukaemic blasts from a child with early thymic precursor (ETP) ALL (Zhang et al, 2012). Diagnostic karyotypes of two T-ALL patients revealed similar t(10;12) translocations (Table 1), leading us to suspect the presence of NAP1L1-MLLT10. Expression of the transcript was confirmed by reverse transcription polymerase chain reaction (RT-PCR) (Fig 1A), and direct sequencing revealed fusion of NAP1L1 exon 14 and MLLT10 exon 4 in both cases (Fig 1B).
Table 1. Clinical details of the **NAP1L1-MLLT10** positive patients. **HOXA9** expression was calculated relative to the housekeeping gene **ABL1**, whereby a ratio of 1 indicated equivalent levels of the two transcripts.

<table>
<thead>
<tr>
<th></th>
<th>Patient A</th>
<th>Patient B</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>16</td>
<td>22</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Male</td>
<td>Male</td>
</tr>
<tr>
<td><strong>WBC count (×10⁹/l)</strong></td>
<td>25·6</td>
<td>297·9</td>
</tr>
<tr>
<td><strong>Immunophenotype</strong></td>
<td>CD34⁻ CD1a⁻ CD3⁻ eCD3⁺ CD5⁺ CD7⁺ CD4weak</td>
<td>CD34⁻ CD1aweak CD2⁻ CD3⁺ CD5⁺ CD7⁺ CD4⁺</td>
</tr>
<tr>
<td></td>
<td>CD8⁻ CD10⁻ TCRAB⁻ TCRGD⁻ MPO⁻</td>
<td>CD8⁻ CD10⁺ TCRGD⁺ CD13⁻ CD33⁻ CD117⁻</td>
</tr>
<tr>
<td><strong>CNS Infiltration</strong></td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td><strong>Karyotype</strong></td>
<td>46,XY,t(10;12)(p11;q14)</td>
<td>46,XY,t(10;12)(p1?2;q14)</td>
</tr>
<tr>
<td><strong>HOXA9/ABL1</strong></td>
<td>3</td>
<td>1·7</td>
</tr>
<tr>
<td><strong>TCR status</strong></td>
<td>Immature, <strong>TRG</strong> rearranged</td>
<td>TCRγδ Positive</td>
</tr>
<tr>
<td><strong>NOTCH1</strong></td>
<td>Wild-type</td>
<td>Mutated</td>
</tr>
<tr>
<td><strong>FBXW7</strong></td>
<td>Mutated</td>
<td>Wild-type</td>
</tr>
<tr>
<td><strong>PTEN/NRAS/KRAS</strong></td>
<td>Wild-type</td>
<td>Not performed</td>
</tr>
<tr>
<td><strong>MRD</strong></td>
<td>Positive</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Clinical course</strong></td>
<td>Persistent MRD positivity (3%) post-induction and consolidation phases of treatment.</td>
<td>Remained in remission from leukaemia but died 2 years post-SCT due to chronic GVHD.</td>
</tr>
</tbody>
</table>
Figure 1. Characterization of the \textit{NAP1L1-MLLT10} translocation in T-ALL. (A) RT-PCR using primers specific for \textit{NAP1L1-MLLT10}. The expected product size of 454 bp is observed for Patient A (Lane 2) and Patient B (Lane 1). Two other patients (Lanes 3 and 4) are negative. NTC, no template control. (B) Confirmation of expression of an in-frame \textit{NAP1L1-MLLT10} fusion transcript by direct (Sanger) sequencing. Exon numbers are indicated. (C) Screening of 565 T-ALL patients for \textit{NAP1L1-MLLT10}. PCR testing was targeted to cases with elevated \textit{HOXA9}. The screening cut-off was based on the lowest \textit{HOXA9/ABL1} associated with a known \textit{HOXA}-activating genetic abnormality (0·5 in a \textit{PICALM-MLLT10} patient). In order not to exclude \textit{NAP1L1-MLLT10} cases that might have lower \textit{HOXA}
expression, the testing threshold was extended to patients with \textit{HOXA9}/\textit{ABL1} \( \geq 0.4 \). Numbers in the relevant groups are indicated. Of the 173 HOXA High cases, 91 had known \textit{HOXA}-activating translocations, and 63 of the remaining 82 had material available for analysis. (D) Taqman Low-Density Array (TLDA) analysis of \textit{HOX} gene transcription in \textit{NAPI1L1-MLLT10} cases. Patient (Pt) samples show specific increases in \textit{HOXA} gene expression relative to three normal thymic (Thy) controls, while other \textit{HOX} loci were not activated.

Clinical details of the \textit{NAPI1L1-MLLT10} patients are shown in Table 1. Strikingly, both were found to have high expression of \textit{HOXA9} by quantitative real time RT-PCR (QRT-PCR). Similar to the index case, Patient A had an ETP-ALL-like immunophenotype, with absence of CD1a, and expression of HLA-DR and the CD13 and CD33 myeloid markers (Table 1). Detailed characterization of T-cell receptor gene configuration revealed biallelic rearrangement of \textit{TRD} and \textit{TRG} and incomplete rearrangement of the \textit{TRB} locus, conforming to an immature pre-\(\beta\)-selection immunogenenotype (Asnafi \textit{et al.}, 2003). In contrast, Patient B exhibited a mature TCR\(\gamma\delta\)+ phenotype and absence of expression of myeloid markers. In common with the reported cases of \textit{DDX3X-MLLT10} T-ALL (Brandimarte \textit{et al.}, 2014), both cases had NOTCH1-activating mutations.

Notably, both patients had poor initial treatment responses, with positive minimal residual disease (MRD). Patient B had persistent MRD positivity throughout induction and consolidation therapy, which necessitated allogeneic stem cell transplantation (allo-SCT) 7 months after diagnosis. Although this resulted in molecular remission, the patient ultimately died of SCT-related complications 2 years later while in remission from leukaemia. The follow-up of Patient A is at an early stage, and latest assessment revealed significant persistent MRD positivity following the consolidation phase.

We sought to determine the incidence of the \textit{NAPI1L1-MLLT10} translocation in T-ALL by screening 565 cases, comprising 141 patients under 16 years of age and 424 adults, using a specific and sensitive (<1%, data not shown) QRT-PCR assay (Fig 1C). As \textit{MLLT10} translocations are normally associated with \textit{HOXA} locus activation (Brandimarte \textit{et al.}, 2013; Bond \textit{et al.}, 2014), we targeted the screening to 173 patients with elevated \textit{HOXA9} at diagnosis (‘HOXA High’ in Fig 1C). We detected no further cases of \textit{NAPI1L1-MLLT10} in patients for whom leukaemic material was available for testing. We therefore estimate the incidence of the translocation in T-ALL to be 0.35% (2/565 patients), and 1.2% of patients with \textit{HOXA9}/\textit{ABL1} \( >0.4 \) (2/173 patients).

\textit{HOXA} overexpression has not previously been described to be associated with \textit{NAPI1L1-MLLT10}. In order to fully characterize \textit{HOX} transcription in these cases, we performed Taqman Low-Density Array (TLDA) analysis of diagnostic RNA. This revealed specific deregulation of \textit{HOXA} gene expression in both samples (Fig 1D). These results, along with the predicted retention of the MLLT10 octapeptide-motif leucine zipper (OM-LZ) domain in the fusion protein, suggest that \textit{NAPI1L1-MLLT10} is likely to recruit the DOT1 Ligand histone methyltransferase and to activate the \textit{HOXA} locus by a similar mechanism as \textit{PICALM-MLLT10} (Okada \textit{et al.}, 2005).

It remains unclear how the activities of \textit{MLLT10} fusion partners might contribute to T-ALL biology. Prediction of \textit{NAPI1L1} (Nucleosome assembly protein 1-like1) function is based on its structural resemblance to \textit{NAP1}, which is thought to be involved primarily in chromatin modulation. \textit{NAP1} has been shown to interact directly with histone H2A-H2B dimers \textit{in vitro} (Okuwaki \textit{et al.}, 2010), suggesting that \textit{NAPI1L1} might alter the regulation of histone
dynamics. It remains to be determined whether the epigenetic effects of NAP1L1 haploinsufficiency and/or activity of a NAP1L1 fusion protein might contribute to a leukaemic phenotype.

In summary, NAP1L1-MLLT10 is a rare recurrent translocation in T-ALL and we report an overall incidence of 0.35%. This may be an underestimation, as our QRT-PCR screening method may not have identified patients with novel breakpoints, and lack of diagnostic material precluded screening in 11% of HOXA-overexpressing patients. Reassuringly, all three cases of NAP1L1-MLLT10 described to date exhibited the t(10;12) translocation by conventional karyotyping, and none of our patient cohort had evidence of chromosome 10 or 12 rearrangement. As with other MLLT10 fusions, NAP1L1-MLLT10 is associated with HOXA deregulation and poor early treatment response. Although one of our patients died of transplant-related complications, the prolonged leukaemia-free survival in this case suggests that these patients should be considered for allo-SCT in first remission, particularly in the event of persistent treatment resistance and/or MRD positivity.

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Conflicts of interest

The authors report no conflicts of interest.

References


