

**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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# 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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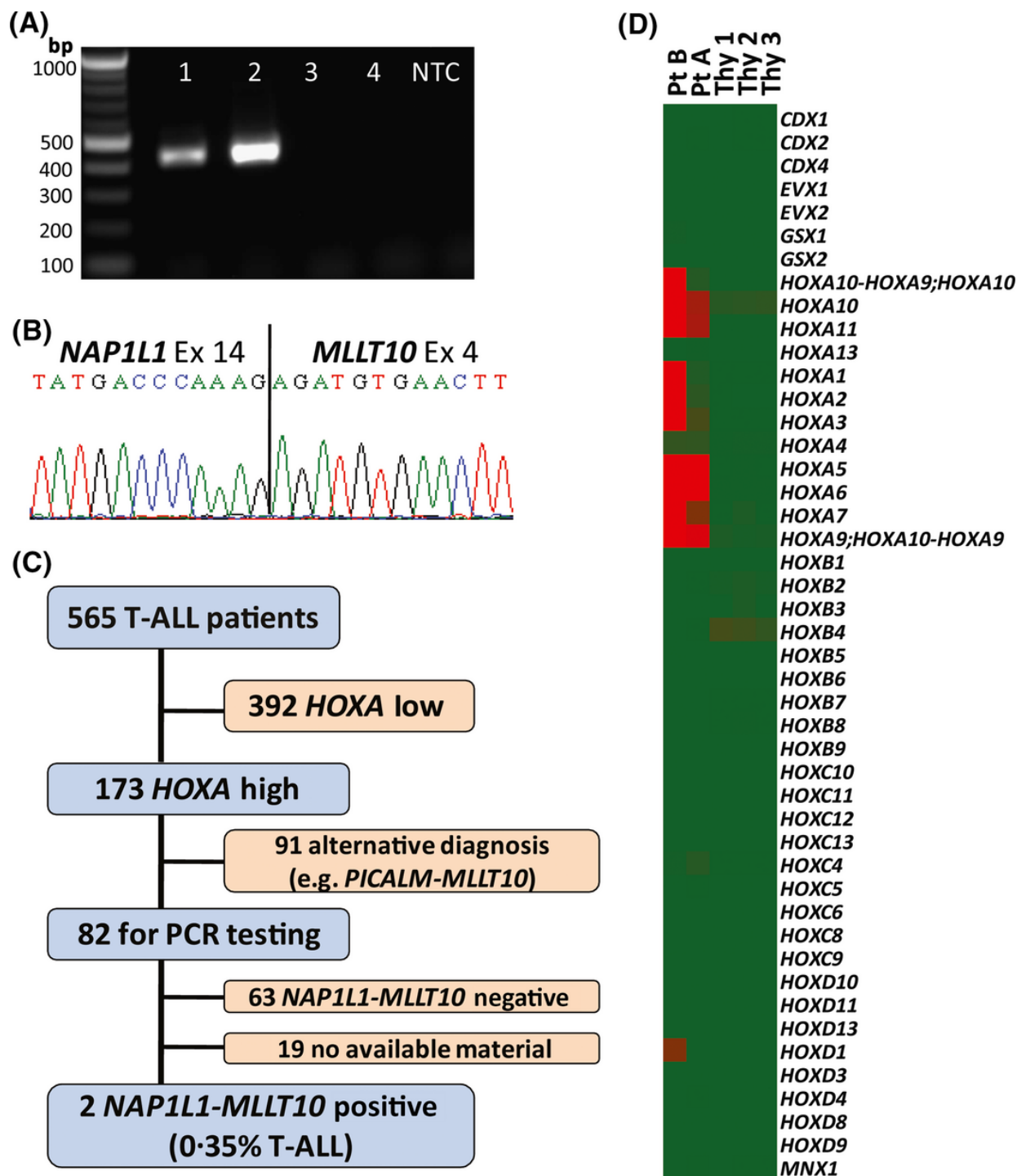
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*MLLT10* (previously *AF10*) was originally described as a fusion partner of the *KMT2A* (previously *MLL*) gene in acute myeloid leukaemia (AML) (Beverloo *et al*, 1995), and was subsequently found to be recurrently rearranged in both AML and T-acute 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

	<b>Patient A</b>	<b>Patient B</b>
1. WBC, white blood cell; TCR, T-cell receptor; <i>TRG</i> , TCR gamma; MRD, minimal residual disease; SCT, Stem cell transplant; GVHD, Graft-versus-host disease.		
Age (years)	16	22
Sex	Male	Male
WBC count ( $\times 10^9/l$ )	25.6	297.9
Immunophenotype	CD34 <sup>-</sup> CD1a <sup>-</sup> CD3 <sup>-</sup> cCD3 <sup>+</sup> CD5 <sup>+</sup> CD7 <sup>+</sup> CD4 <sup>weak</sup>	CD34 <sup>-</sup> CD1a <sup>weak</sup> CD2 <sup>-</sup> CD3 <sup>+</sup> CD5 <sup>+</sup> CD7 <sup>+</sup> CD4 <sup>+</sup>
	CD8 <sup>-</sup> CD10 <sup>-</sup> TCRAB <sup>-</sup> TCRGD <sup>-</sup> MPO <sup>-</sup>	CD8 <sup>-</sup> CD10 <sup>+</sup> TCRGD <sup>+</sup> CD13 <sup>-</sup> CD33 <sup>-</sup> CD117 <sup>-</sup>
	CD117 <sup>-</sup> CD13 <sup>+</sup> CD33 <sup>+</sup> HLA-DR <sup>+</sup>	
CNS Infiltration	No	No
Karyotype	46,XY,t(10;12)(p11;q14)	46,XY,t(10;12)(p1?2;q14)
<i>HOXA9/ABL1</i>	3	1.7
TCR status	Immature, <i>TRG</i> rearranged	TCR $\gamma\delta$ Positive
<i>NOTCH1</i>	Wild-type	Mutated
<i>FBXW7</i>	Mutated	Wild-type
<i>PTEN/NRAS/KRAS</i>	Wild-type	Not performed
MRD	Positive	Positive
Clinical course	Persistent MRD positivity (3%) post-induction and consolidation phases of treatment.	Underwent SCT 7 months post- diagnosis. Remained in remission from leukaemia but died 2 years post-SCT due to chronic GVHD.



**Figure 1.** Characterization of the *NAP1L1-MLLT10* translocation in T-ALL. (A) RT-PCR using primers specific for *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 *NAP1L1-MLLT10* fusion transcript by direct (Sanger) sequencing. Exon numbers are indicated. (C) Screening of 565 T-ALL patients for *NAP1L1-MLLT10*. PCR testing was targeted to cases with elevated *HOXA9*. The screening cut-off was based on the lowest *HOXA9/ABL1* associated with a known *HOXA*-activating genetic abnormality (0.5 in a *PICALM-MLLT10* patient). In order not to exclude *NAP1L1-MLLT10* cases that might have lower *HOXA*

expression, the testing threshold was extended to patients with  $HOXA9/ABL1 \geq 0.4$ . Numbers in the relevant groups are indicated. Of the 173 HOXA High cases, 91 had known HOXA-activating translocations, and 63 of the remaining 82 had material available for analysis. (D) Taqman Low-Density Array (TLDA) analysis of HOX gene transcription in *NAP1L1-MLLT10* cases. Patient (Pt) samples show specific increases in HOXA gene expression relative to three normal thymic (Thy) controls, while other HOX loci were not activated.

Clinical details of the *NAP1L1-MLLT10* patients are shown in Table 1. Strikingly, both were found to have high expression of 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 *TRD* and *TRG* and incomplete rearrangement of the *TRB* locus, conforming to an immature pre- $\beta$ -selection immunogenotype (Asnafi *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 *DDX3X-MLLT10* T-ALL (Brandimarte *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 *NAP1L1-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 *MLLT10* translocations are normally associated with HOXA locus activation (Brandimarte *et al*, 2013; Bond *et al*, 2014), we targeted the screening to 173 patients with elevated HOXA9 at diagnosis ('HOXA High' in Fig 1C). We detected no further cases of *NAP1L1-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  $HOXA9/ABL1 > 0.4$  (2/173 patients).

HOXA overexpression has not previously been described to be associated with *NAP1L1-MLLT10*. In order to fully characterize HOX transcription in these cases, we performed Taqman Low-Density Array (TLDA) analysis of diagnostic RNA. This revealed specific deregulation of 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 NAP1L1-MLLT10 is likely to recruit the DOT1 Ligand histone methyltransferase and to activate the HOXA locus by a similar mechanism as PICALM-MLLT10 (Okada *et al*, 2005).

It remains unclear how the activities of *MLLT10* fusion partners might contribute to T-ALL biology. Prediction of NAP1L1 (Nucleosome assembly protein 1-like1) function is based on its structural resemblance to NAP1, which is thought to be involved primarily in chromatin modulation. NAP1 has been shown to interact directly with histone H2A-H2B dimers *in vitro* (Okuwaki *et al*, 2010), suggesting that NAP1L1 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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JB, AT, AC, AT, MM and TF performed and interpreted diagnostic investigations. TM, ME, AC and CS provided clinical care and interpreted clinical data. JB, VA and EAM analysed and collated data and wrote the manuscript. JB is supported by a Kay Kendall Leukaemia Fund Intermediate Research Fellowship. The Macintyre laboratory is supported by the Association Laurette Fugain and the INCa CAMELE Translational Research and PhD Programmes.

## Conflicts of interest

The authors report no conflicts of interest.

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