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

	Patient A	**Patient B**
Age (years)	16	22
Sex	Male	Male
WBC count ($\times 10^9$/l)	25·6	297·9
Immunophenotype	$CD34^-$ $CD1a^-$ $CD3^-$ $cCD3^+$ $CD5^+$ $CD7^+$ CD4weak $CD8^-$ $CD10^-$ TCRAB$^-$ TCRGD$^-$ MPO^- $CD117^-$ $CD13^+$ $CD33^+$ HLA-DR^+	$CD34^-$ CD1aweak $CD2^-$ $CD3^+$ $CD5^+$ $CD7^+$ $CD4^+$ $CD8^-$ $CD10^+$ TCRGD$^+$ $CD13^-$ $CD33^-$ $CD117^-$
CNS Infiltration	No	No
Karyotype	46,XY,t(10;12)(p11;q14)	46,XY,t(10;12)(p1?2;q14)
HOXA9/*ABL1*	3	1·7
TCR status	Immature, *TRG* rearranged	TCRγδ Positive
NOTCH1	Wild-type	Mutated
FBXW7	Mutated	Wild-type
PTEN/NRAS/KRAS	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.

1. WBC, white blood cell; TCR, T-cell receptor; *TRG*, TCR gamma; MRD, minimal residual disease; SCT, Stem cell transplant; GVHD, Graft-versus-host disease.

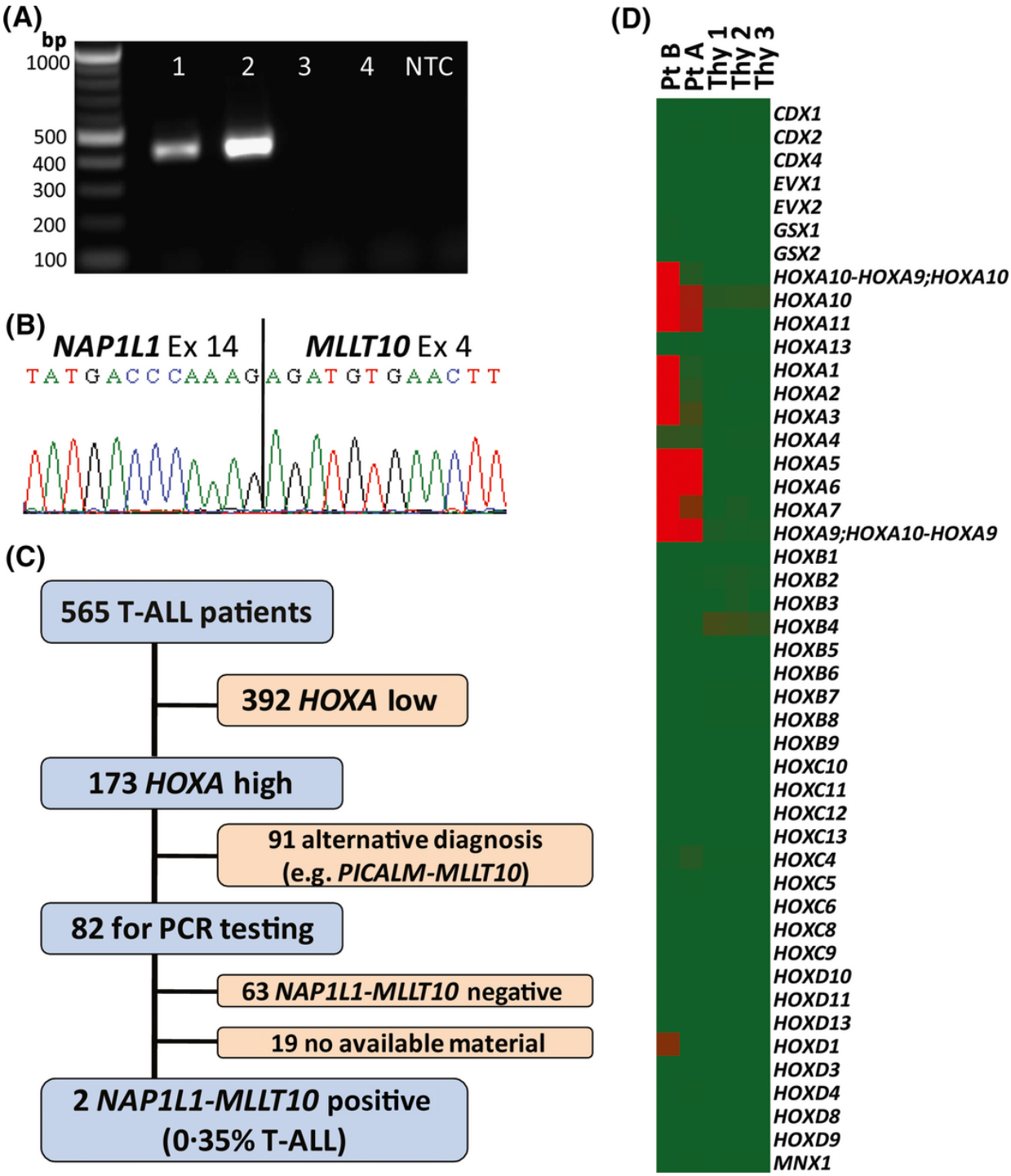

Figure 1. Characterization of the *NAP1L1-MLLT10* translocation in T-ALL. (A) RT-PCR using primers specific for *NAP1L1-MLLT10*. The expected product size of 454 bp is observed for Patient A (Lane 2) and Patient B (Lane 1). Two other patients (Lanes 3 and 4) are negative. NTC, no template control. (B) 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* ≥ 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-β-selection immunogenotype (Asnafi *et al*, 2003). In contrast, Patient B exhibited a mature TCRγδ+ 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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Conflicts of interest

The authors report no conflicts of interest.

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