Whole transcriptome sequencing of a paediatric case of *de novo* acute myeloid leukaemia with del(5q) reveals *RUNX1-USP42* and *PRDM16-SKI* fusion transcripts

Fusion genes are frequently detected in childhood acute myeloid leukaemia (AML) and other haematopoietic cancers. Recent decades have witnessed the identification, through chromosomal analysis techniques or reverse transcription polymerase chain reaction (RT-PCR), of several fusion genes influencing either proliferation/apoptosis or differentiation ability of AML cells (Gianfelici *et al*, 2012). The advent of next generation sequencing (NGS) has dramatically improved our ability to investigate the mutational profile of cancer cells, and several studies used NGS to identify novel mutations and chromosomal alterations in haematological neoplasms (Masetti *et al*, 2013). Along this line of research, we performed whole-transcriptome sequencing (WTS) of blast cells from a paediatric AML patient with del(5q) to elucidate the mutational profile of this rare type of childhood leukaemia associated with resistance to chemotherapy and grim survival (Van den Berghe *et al*, 1985).

An 8-year-old child was diagnosed with AML French-American-British (FAB) type M5b. Clinical presentation was characterized by hyperleucocytosis (96·18 x 10^9/l), anaemia (7·6 g/l) and thrombocytopenia (105 x 10^9/l). Bone marrow (BM) smears showed 60% blasts; conventional cytogenetics on BM cells revealed an abnormal karyotype, 46, XY, del(5)(q14q34), in 20 metaphases. No recurrent genetic abnormality involving *KMT2A* (also termed *MLL*), *CBFB*, *NPM1* and *FLT3* genes was found. The patient was enrolled in the Associazione Italiana di Ematologia e Oncologia Pediatrica (AIEOP) AML 2002/01 Protocol. After first induction course, BM evaluation on day +21 documented induction failure (88% blasts). Two cycles of salvage treatment allowed the patient to achieve complete remission. Thereafter, he received allogeneic haematopoietic transplantation from a human leucocyte antigen (HLA)-identical unrelated donor. 5 years after the allograft, the patient is alive and disease-free.

The massively parallel sequencing of the transcriptome allowed us to map 50·3 million reads at a mean depth of 20X. Giving value to only concordant results emerging from the use of three distinct algorithms, namely Chimerascan, deFuse and FusionMap, two fusion transcripts, *RUNX1-USP42* and *PRDM16-SKI*, were identified in the patient. Perusal of the reads mapping these chimeric transcripts showed that the first was an in-frame fusion, while *PRDM16-SKI* was a novel out-of-frame fusion (Fig 1A). RT-PCR analysis and Sanger sequencing confirmed the presence of both chimeric transcripts (Fig 1B). To date, the cryptic t(7;21) leading to the fusion between *RUNX1* and *USP42* has been reported only once in children (Paulsson *et al*, 2006), while seven adult cases of myeloid neoplasms harbouring the *RUNX1-USP42* transcript have been published (Table I), suggesting that this genetic abnormality is a rare, but non-random, feature of myeloid malignancies in adults. To assess the recurrence of *RUNX1-USP42* fusion transcript in childhood AML, we examined 132 children with cytogenetically normal (CN) *de novo* AML enrolled in the AIEOP AML 2002/01 Protocol. No additional patient was found to carry this chimeric transcript.

To the best of our knowledge, this is the second paediatric case in which the *RUNX1-USP42* fusion transcript has been detected and, unprecedentedly, it was observed at diagnosis together with del(5q), whereas in the case described by Paulsson *et al* (2006) the 5q- occurred later in the disease course. In our case, the fusion occurred between exon 6 of *RUNX1* and exon 3 of *USP42* (Fig 1A-B). However, as previously reported, owing to different breakpoints and alternative splicing in *RUNX1*, several splice-variants of *RUNX1-USP42* chimeric transcript may be generated by the cryptic t(7;21) (Foster *et al*, 2010) (Table I). This notwithstanding, all *RUNX1-USP42* isoforms harbour both the catalytic domain (UCH) of *USP42* and the highly conserved Runt homology domain (RHD) of *RUNX1*, which mediates DNA binding and heterodimerization of RUNX1 with CBFβ. Several mechanisms through which this chimeric transcript could contribute to the leukaemogenesis have been proposed: (i) dominant negative inhibition of wild-type *RUNX1* transcription activation activity (Paulsson *et al*, 2006; Panagopoulos *et al*, 2013), (ii) *USP42*-mediated stabilization of *RUNX1* from ubiquitin-proteasome degradation (Paulsson *et al*, 2006), and iii) ectopic over-expression of *USP42* and deregulation of TP53-dependent cell-cycle arrest (Giguère & Hébert, 2011). In accordance with the last hypothesis, through RT-qPCR analysis, we demonstrated an over-expression of *USP42* in t(7;21)-positive cells when compared with other CN-AML cells lacking this translocation, and CD34+ haematopoietic progenitors ($P = 0·02$) (Fig 1C). This finding was further validated through gene expression analysis performed on the WTS data (Fig 1D). Taken together, these

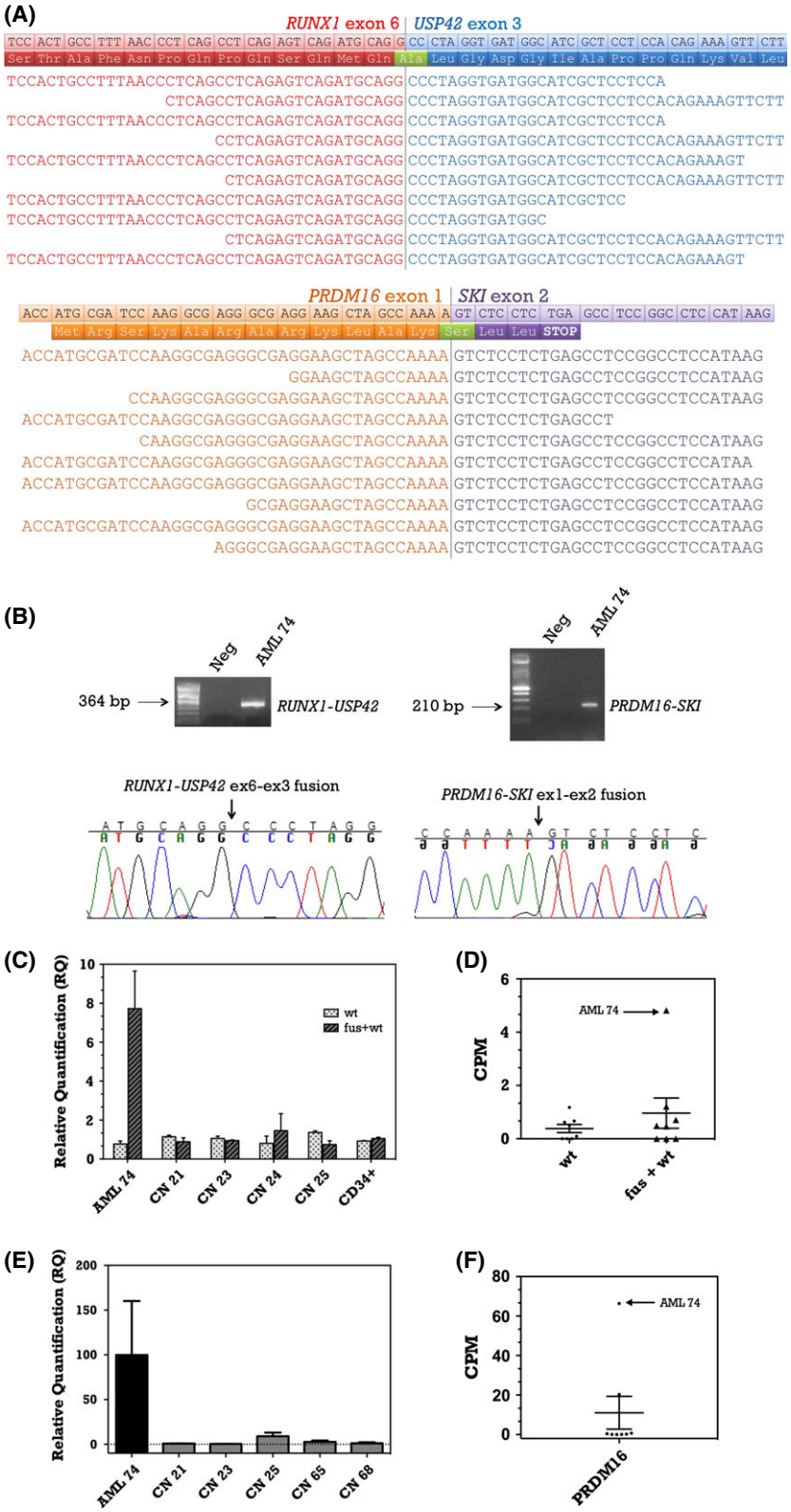

Fig 1. Chimeric transcripts identified through WTS in a child with acute myeloid leukaemia (AML) and del(5q). (A) Schematic representation of split read mapping the *RUNX1-USP42* and *PRDM16-SKI* fusions obtained by whole-transcriptome sequencing (WTS). Defuse, Chimerascan, and FusionMap packages were used to detect chimeric transcripts from RNA-seq data as previously described (Masetti *et al*, 2013). Predicted sequences of the chimeric proteins are reported. (B) Reverse transcription polymerase chain reaction (RT-PCR) analysis and Sanger sequencing were performed in order to validate the detection of *RUNX1-USP42* and *PRDM16-SKI* fusions. Neg = negative. (C) Quantitative RT-PCR (qRT-PCR) analysis was performed in order to detect differential expression of the *USP42* gene. As a result of the t(7;21) translocation, *USP42* gene is placed under the control of the *RUNX1* promoter, and this could lead to over-expression of the rearranged *USP42* gene. To test this possibility, we designed a RT-qPCR with two different primer pairs (wild-type [wt]: primers mapping in exons 2–3 of *USP42*; and wt+fusion [fus]: primers mapping in exons 7–8 of *USP42*) and we evaluated the expression of the wt and the wt+rearranged *USP42* in 4 cytogenetically normal AML (CN-AML) samples (CN21, CN23, CN24, CN25), the t(7;21)(p22;q22) positive case (AML74) and in $CD34^+$ haematopoietic stem cells. (D) Expression levels of *USP42* obtained from WTS data, as described previously (Masetti *et al*, 2013), in the t(7;21)(p22;q22) positive patient (AML74), and 7 CN-AML patients (CN21, CN22, CN23, CN24, CN25, CN65, CN68). CPM = count per million. (E) qRT-PCR analysis was performed in order to detect differential expression of the *PRDM16* gene in the t(7;21)(p22;q22) positive case (AML74), and in five paediatric CN-AML samples (CN21, CN23, CN25, CN65, CN68). (F) Expression levels of *PRDM16* obtained from WTS data, as described previously (Masetti *et al*, 2013), in the t(7;21)(p22;q22) positive patient (AML74), and 7 CN-AML patients (CN21, CN22, CN23, CN24, CN25, CN65, CN68).

results confirm and extend previous reports (Panagopoulos *et al*, 2013), underlining once again the potential role of this proteinase in leukaemogenesis, and lending support to both the role of deubiquitinating proteins in tumourigenesis and the use of deubiquitinating enzyme inhibitors in cancer therapy.

Table I. Main characteristics of t(7;21)(p22; q22) positive patients.

Patient	Age, years	Gender	Disease	WBC, x 10^9/l	%BM blasts at diagnosis	del(5q) at diagnosis	Fusion (isoform)	HSCT (type)	Relapse	Reference
1	7	Male	AML M0	35·6	75–80%	No	RUNX1ex7(iso2)-USP42ex3 (iso1)	Yes (SIB)	Yes	Paulsson *et al* (2006)
2	68	Female	MDS (RAEB-2)	NA	17%	No	RUNX1ex6 (iso2)-USP42ex3 (iso1)	No	No	Foster *et al* (2010)
3	68	Male	AML M5	NA	NA	No	RUNX1ex7 (iso2)-USP42ex3 (iso1)	No	No	Foster *et al* (2010)
4	32	Male	AML M1	12·1	90%	Yes	RUNX1ex7 (iso2)-USP42ex3 (iso1)	NA	NA	Giguère and Hébert (2011)
5	54	Male	AML M4/M5	17·6	73%	Yes	RUNX1ex7 (iso2)-USP42ex3 (iso1)	Yes (NA)	Yes	Jeandidier *et al* (2012)
6	33	Male	AML M5a	5·4	84%	Yes	RUNX1ex7 (iso2)-USP42ex3 (iso1)	Yes (SIB)	No	Jeandidier *et al* (2012)
7	39	Male	AML M4/M5	1·3	NA	Yes	RUNX1ex6 (iso2)-USP42ex3 (iso1)	No	NA	Jeandidier *et al* (2012)
8	52	Female	AML M0	34	>70%	Yes	RUNX1ex7 (iso2)-USP42ex3 (iso1)	Yes (SIB)	No	Panagopoulos *et al* (2013)
9	8	Male	AML M5	96·18	60%	Yes	RUNX1ex6 (iso2)-USP42ex3 (iso1)	Yes (MUD)	No	Present report

WBC, white blood cell count; BM, bone marrow; HSCT, haematopoietic stem cell transplantation; AML, acute myeloid leukaemia; MDS, myelodysplastic syndrome; RAEB-2, refractory anaemia with excess blasts, type 2; SIB, sibling; MUD, matched unrelated donor; NA, not available.

Animal models have shown that *RUNX1*-related translocations or haploinsufficiency of *RUNX1* are not sufficient for leukaemogenesis; additional genetic events are required. Interestingly, WTS enabled the indentification of a novel out-of-frame fusion transcript in which exon 1 of *PRDM16* (also known as *MEL1*) is fused with exon 2 of *SKI* (*v-ski* avian sarcoma viral oncogene homolog). Due to loss of the open-reading frame, no putative chimeric protein seems to be encoded by this fusion transcript. However, considering the relative position of the two genes on the forward strand of chromosome 1, we speculate that the juxtaposition of *SKI* downstream of exon 1 of *PRDM16* may lead to slipping of *PRDM16* and this, in turn, positions the gene under the control of ectopic promoter/enhancer elements (Fig 1B). To test this hypothesis, we evaluated the expression levels of *PRDM16* by RT-qPCR and gene expression analysis on WTS data, and found that its expression was significantly enhanced in the t(7;21)-positive patient compared to other CN-AML patients ($P = 0{\cdot}01$) (Fig 1E and F). Additionally, *SKI* expression was investigated through both RT-qPCR and WTS gene expression analysis. However, no differential expression of this gene was detected (data not shown). Coding for a zinc-finger protein containing a DNA-binding PRDI-BF1/RIZ homologous (PR) domain, *PRDM16* belongs to the *EVI1* family, and is implicated in two translocations involving *RUNX1* and *RPN1* in myeloid malignancies. Interestingly, several reports indicate the link between overexpression of *PRDM16* and leukaemogenesis, strengthening its association with a worse outcome (Nishikata *et al*, 2003; Duhoux *et al*, 2012).

In summary, we report, for the first time, the identification of a *RUNX1-USP42* fusion transcript detected at diagnosis in a child with AML and del(5). No other child was positive for this chimeric transcript in a validation cohort, demonstrating that this is a rare genetic lesion in childhood AML. Interestingly, WTS also enabled the identification of a novel out-of-frame *PRDM16-SKI* fusion, and analysis of *PRDM16* revealed an over-expression of this gene.

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Authorship contributions

R.M. and M.T. coordinated the work, analysed data, performed statistical analyses and wrote the paper. M.T. and A.A. performed the whole-transcriptome massively parallel sequencing. V.I. performed bioinformatics analyses. M.P. and E.M. performed the screening in the validation cohort. B.R. analysed clinical data and wrote the paper. G.B., A.P. and F.L. designed and supervised the research. S.R. and F.L. equally contributed to the critical revision and writing of the

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Conflicts of interest

The authors declare no competing financial interest.

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Regulatory T-cell depletion in donor lymphocyte infusions for haematological malignancies: long-term outcomes from a prospective study

Donor lymphocyte infusion (DLI) is routinely used for patients who relapse after allogeneic haematopoietic stem cell transplantation (HSCT) but is often unsuccessful in controlling malignancy (Schmid *et al*, 2007). The regulatory T-cells (Treg) fraction contained within donor T-cells may reduce alloreactivity, the principal component of the graft-*versus*-tumour (GVT) effect (Cohen *et al*, 2002). Hypothesizing that Treg depletion could improve alloreactivity and, consequently, the GVT effect of DLI, we previously developed a multi-institutional phase I/II clinical trial to investigate Treg