

SQSTM1-NUP214: a new gene fusion in adult T-cell acute lymphoblastic leukemia

In patients with T-cell lymphoblastic leukemia (T-ALL) chromosome rearrangements and gene mutations are used as diagnostic markers for genetic classification and prognostic stratification.¹ Gene deregulation in T-ALL is determined by ectopic or over-expressed oncogenes, gain or loss of function mutations, genomic imbalances and, less frequently, gene fusions. In T-ALL rare fusions involving 2 nucleoporins, i.e. *NUP98* (*NUP98-RAP1GDS1*, *NUP98-SETBP1*, *NUP98-C6orf80*) and *NUP214* (*NUP214-ABL1* and *SET-NUP214*), are believed to predict a poor prognosis.²

We found a *SET-NUP214* rearrangement was closely associated with a specific gene profiling signature in adult T-ALL³ and analysis of 69 patients who were enrolled in 3 Italian GIMEMA protocols (LAL0496, LAL2000 and LAL0904⁴) revealed one case had a slightly different profile.

Here we report, for the first time, the case of a 20-year old man, with a chemoresistant pre-T ALL (immunophenotype: CD3⁺, CD7⁺, CD5⁺, CD34⁺, CD33⁺, cKit⁺) who died 16 months after diagnosis. The karyotype was 46,XY,del(6p)(p21p25) and CI-FISH detected *CDKN2A-B/9p21* and *NF1/17q11* deletions.⁵

Using the list of genes that were specific for *SET/NUP214*+ cases,³ this patient clustered tightly with this group and showed high expression levels of *HOXA* cluster genes (*HOXA3*, *HOXA5*, *HOXA7*, *HOXA9*, *HOXA10*) and *NUP214* as well as low expression of *FNBP1*, *C9orf78* and *USP20* but unlike them, he showed remarkably high *SET* levels (Figure 1A).

Consequently, we investigated *NUP214* and found it was involved in a cryptic unbalanced translocation. FISH with RP11-143H20, flanking *NUP214* at the centromeric side, hybridized with both normal chromosomes 9, as expected, while RP11-544A12 spanning *NUP214*, hybridized with both 9 and an apparently normal chromosome 5. To characterize the rearrangement between chromosome 9 and 5 we used DNA clones for chromosome bands 9q34 and 5q35 and found a trisomic 9q34-qter underwent an unbalanced translocation with chromosome 5, i.e. der(5)t(5;9)(q35;q34) (Figure 1B and C). The 9q34/*NUP214* breakpoint was narrowed using two overlapping clones. Clone G248P89801E11 spanning exons 25-32 was detected on two normal chromosomes 9, while clone G248P8659A12 spanning exons 31-36 was present on chromosomes 9 and on der(5) (Figure 1B and C). Thus the breakpoint fell within 3' region of *NUP214*.

The 5q35 breakpoint was telomeric to RP11-718N2, a ~1.7Mb region containing 15 candidate genes, with an appropriate centromere-telomere orientation. Since GEP revealed 3/15 genes telomeric to *SQSTM1/5q35* were down-modulated, they might have been lost as a result of unbalanced der(5)t(5;9). *SQSTM1* was first selected as putative *NUP214* fusion partner.

Using primers *SQSTM1_ex3_528F* (5'-TGCCAGAC-TACGACTTGTG-3') and *NUP214_ex36_6543R* (5'-AGTAATCATGCGCCTTGTGAGTT-3'), RT-PCR detected an amplification product of 852bp which was sub-cloned into pGEM-T easy vector (Promega, Madison, WI, USA). Sequencing showed an in-frame fusion between nucleotide 849 (exon 5) of *SQSTM1* and nucleotide 6014 (exon 33) of *NUP214* (Figure 2A and B). To establish the prevalence more accurately we screened another 67 T-ALL patients.

We set up a Nested RT-PCR assay, using primers *SQSTM1_ex3_528F* and *NUP214_ex36_6543R* in the first amplification round and primers *SQSTM1_ex4/5_763F* (5'-AATCAGCTTCTGGTCCATCG-3') and *NUP214_ex_33/34_6337R* (5'-CAAAGCTGAACCCTC-CTGTG-3') in the second. Nested RT-PCR were also used to validate 15/69 cases analyzed by GEP for which cDNA

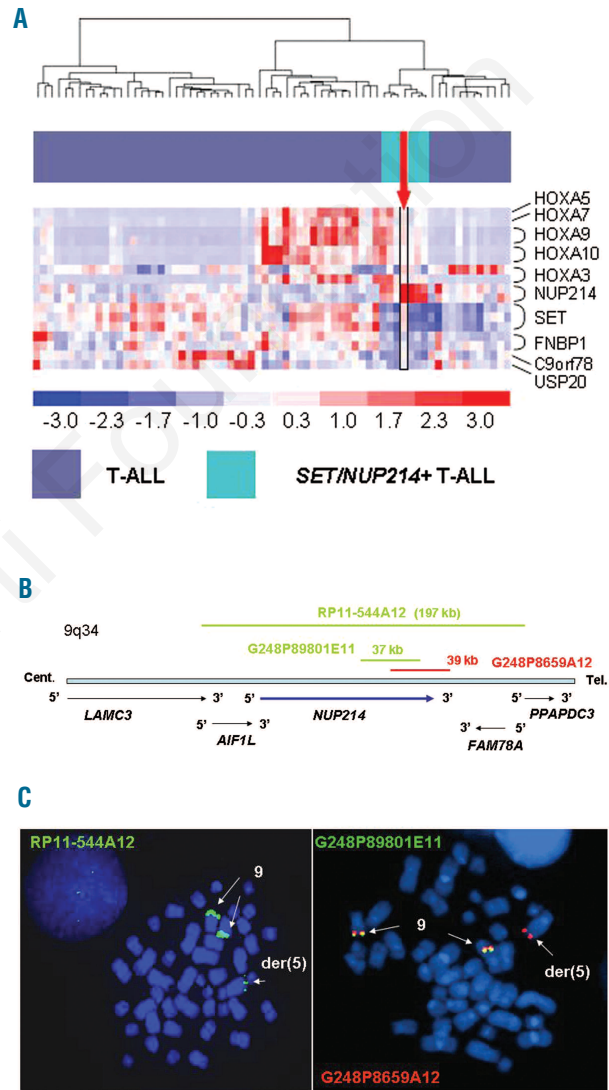


Figure 1. Gene expression profiles and FISH assays. (A) Identification of additional *SET/NUP214*+ patients by using the genes specific of *SET/NUP214*+ signature. Each row represents a probe-set, each column a sample. Color scale: blue and red indicate the lowest and highest expression levels, respectively. The reported case is indicated as an arrow: it clustered with the *SET/NUP214*+ patients and was characterized by the upregulation of *HOXA* cluster genes and *NUP214* as well as low expression of *FNBP1*, *C9orf78* and *USP20* while it showed remarkably high *SET* levels. (B) Schema of part of chromosome 9 long arm (9q34). DNA clones used for FISH studies are shown with their relative position and size. Centromere to telomere portion of 9q34 indicates gene localization /orientation (arrows). (C) Metaphase FISH results: (left panel) Clone RP11-544A12 (green), hybridized with both chromosomes 9 and one apparently normal chromosome 5 (der(5)). (Right panel) Clone G248P89801E11 (green) (*NUP214*, exon 25-32) was present on both chromosomes 9. G248P8659A12 (red) (*NUP214*, exons 31-36) was detected on chromosomes 9 and on der(5).

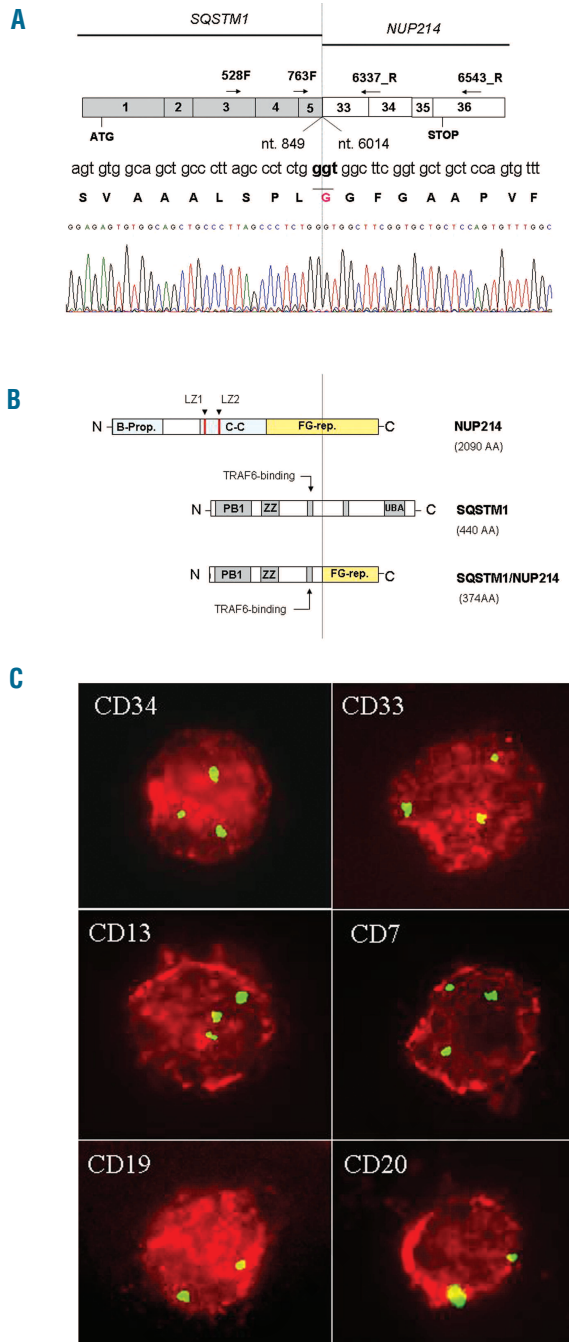


Figure 2. Molecular characterization of *SQSTM1-NUP214* fusion transcript and FICTION experiments. (A) Schema of *SQSTM1-NUP214* fusion transcript. *SQSTM1* nucleotide 849 (exon 5) is fused in-frame to *NUP214* nucleotide 6014 (exon 33). Arrows indicate the primers used in PCR amplification. Dotted line indicates fusion point. Sequence numbers refer to GenBank accession NM_003900.4 for *SQSTM1* and NM_005085.2 for *NUP214*. (B) Schema of *NUP214* (2090 AA) (upper), *SQSTM1* (440 AA) (middle) and the predicted *SQSTM1/NUP214* fusion protein (374 AA) (bottom) which contains PB1, ZZ and TRAF6 binding domain derived from *SQSTM1* and 14/44 FG repeats derived from *NUP214*. The FG repeat region is indicated in yellow. B-prop: Beta propeller, C-C: Coiled-coil, LZ: Leucine-zipper, FG-rep: Phenylalanine-Glycine repeats, PB1: Phox and Bem1p domain, ZZ: Zinc finger, UBA: ubiquitin associated domain. (C) FICTION with monoclonal antibodies against CD34, CD33, CD13, CD7, CD19, CD20. Red staining detects positive intact cells expressing the specific antigen. Green spots indicate FISH signals in the nuclei using a FITC-labeled genomic probe (RP11-544A12) for *NUP214*. Two green signals indicate normal cells; three signals hallmark *NUP214* rearrangement.

was available. PCR screening did not identify any other cases with the *SQSTM1-NUP214* fusion transcript so the prevalence at present is one in 136 cases.

To determine the hematopoietic lineage of this fusion gene we applied FICTION (Fluorescence Immunophenotype and Interphase Cytogenetics as a Tool for Investigations of Neoplasms)⁶ with RP11-544A12 and found the *NUP214* rearrangement in CD34⁺, CD33⁺, CD13⁺, CD14⁺, CD3⁺ and in CD7⁺ bone marrow cells (range of positive cells 50-78%) but not in CD19⁺ and CD20⁺ cells (Figure 2C). FICTION results are concordant to the *ex vivo* immunophenotype, including CD34 positivity. The present case of *SQSTM1-NUP214* positive T-ALL shared a few common features with *SET-NUP214* positive adult T-ALL:³ immature phenotype and poor disease outcome; additional genomic lesions at karyotype and/or CI-FISH; deregulation of the same set of genes, as shown by GEP.

NUP214 maps on chromosome 9q34.3; with its 36 exons, it covers 108 kb of genomic DNA. The transcript is a 6.6 kb long mRNA which encodes for 214 kDa FXFG nucleoporin of 2090 AA located mainly on the cytoplasmic side of the nuclear pore complex.²

SQSTM1 gene, at chromosome 5q35, encodes a multi-functional adaptor protein that has no intrinsic enzymatic activity but is a critical component of multiple signaling pathways. It is mainly known as an ubiquitin binding protein and a scaffolding/adaptor factor that is involved in NF- κ B activation in response to Ras induction.⁷ *SQSTM1* regulates bone remodeling by controlling osteoclastogenesis, inflammatory reaction through T-cell differentiation, NGF receptor internalization and adipocyte differentiation.⁷ Remarkably, *SQSTM1* mutations result in sporadic and familial Paget's disease of bone⁸ and its overexpression has been found in several solid human tumors.⁷ It is involved in tumor growth and bone destruction in patients with multiple myeloma.⁹

In the predicted *SQSTM1-NUP214* fusion protein, as in the *SET-NUP214*, the fusion partner N-terminal links to the *NUP214* C-terminal (Figure 2B). The *SET-NUP214* fusion retains 42/44 *NUP214* FG repeats with the ability to bind and relocalize the NES receptor hCRM1, thus perturbing nuclear transport.¹⁰ *SQSTM1/NUP214* contained only 14/44 FG repeats and since it had not maintained the entire hCRM1 binding domain¹¹ other mechanisms might be implicated in the leukemogenic process.

In conclusion, this case illustrates a cryptic unbalanced translocation der(5)t(5;9)(q35;q34) which underlay a new *SQSTM1-NUP214* in-frame fusion which joined the *SQSTM1* N-terminal with the *NUP214* C-terminal. The prevalence at present is one in a total of 136 cases; 69 in the GEP series⁴ and 67 screened in the present report by RT-PCR.

As *NUP214* behaves as a promiscuous gene undergoing different types of rearrangements in T-ALL, we strongly recommend applying a specific FISH assay to detect *NUP214* in the diagnostic work up of T-ALL. The incidence and clinical impact of *NUP214*-rearrangements need to be investigated in large prospective studies.

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