## Molecular evaluation of the NUP98/RAP1GDS1 gene frequency in adults with T-acute lymphoblastic leukemia

The NUP98/RAP1GDS1 (NRG) is a new fusion gene, originating from the t(4;11)(q21;p15) translocation, that characterizes a subset of T-cell acute lymphoblastic leukemia (T-ALL). In this study we analyzed 43 T-ALL patients for the expression of this new molecular marker using a reverse transcription-polymerase chain reaction (RT-PCR) system, which is more sensitive and specific than cytogenetics alone, confirming that NRG-positive ALLs are infrequent, accounting for approximately 5% of cases.

Hussey *et al*.[1] have recently identified the NUP98/RAP1GDS1 (NRG) as a new hybrid transcript gene originating from the t(4;11)(q21;p15) translocation which fuses the 5' end of the NUP98 gene, at the 11p15 cytogenetic band, in frame to the coding region of the RAP1GDS1, at the 4q21 cytogenetic band. This rare cytogenetic alteration was originally reported in a case of acute lymphoblastic leukemia (ALL), which co-expressed T- and myeloid-associated markers.[2] Following this early observation, seven additional cases were found to show the t(4;11)(q21;p15).[1–5] Altogether, these observations support the specific association of t(4;11)(q21;p15) with T-ALL showing a variable co-expression of CD10 and myeloid markers. In a more recent study of 68 T-ALL cases enrolled in the GIMEMA 0496 protocol, Mecucci *et al*.[5] found an overall 2.9 % frequency of t(4;11) (q21;p15) in the entire T-ALL series analyzed (2/68), which increased to 5.7% in cases for which there were metaphases (35 cases). Moreover, since in the second case of this report the t(4;11)(q21;p15) alteration was cytogenetically detectable only at the time of relapse, because, at that time, the patients' diagnostic sample was not provided by the peripheral institution, it was recommended that the prevalence of this new genetic marker was defined with more accuracy than that achieved by cytogenetics alone. Thus, we have now extended the RT-PCR analysis of NRG transcript to mRNA samples collected at the onset of disease from 43 T-ALL patients enrolled in the GIMEMA 0496 ALL trial,[6] including the t(4;11)(q21;p15) positive cases described by Mecucci *et al*.[5] The specific NUP98/RAP1GDS and RAP1GDS/NUP98 fusion transcripts were amplified by RT-PCR using the oligonucleotide sets kindly provided by M. Negrini, as previously described.[5] The quality of RNA was assessed on an ethidium bromide-stained 1% agarose gel containing 2.2 mol of formaldehyde per liter. Amplification of the normal RARα gene was done with the same cDNA preparation and using PCR reagents and conditions described elsewhere.[7] The NUP98/ RAP1GDS1 chimeric transcript was diagnosed in two out of the 43 T-ALL. Both cases also expressed the reciprocal RAP1GDS1/NUP98 fusion product. As shown in Table 1, cytogenetic analyses, available in 18/43 patients (43%), demonstrated a t(4;11)(q21p15) at diagnosis in one of the NRG-positive patients, whereas, as already reported, the other patient had a normal karyotype at diagnosis and became t(4;11) positive at relapse.

Taken together, these data demonstrate that, even using a sensitive RT-PCR technique, NUP98/RAP1GDS1 positive T-ALL are infrequent, representing no more than 5% of cases of adult T-ALL. Moreover, we have also demonstrated the NRG chimeric transcript in the diagnostic mRNA sample of the patient without evidence of t(4;11) translocation at onset of disease. This observation confirms the notion that, in ALL patients, genetic aberrations are not always identified at the karyotypic level because of the low cycling rate of lymphoid blasts. Alternatively, it is possible that the t(4;11)(q21p15) arose in a minor subclone that, in this patient, became cytogenetically detectable only at relapse, as was suggested to have occurred in an old case of an infant leukemia of uncertain classification in which a few metaphases showed the t(4;11)(q21p15) in addition to the typical t(4;11) involving the 11q23 alteration in all cells.[8]

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**Table 1. Clinico-biological features of the 43 T-ALL patients evaluated for presence of NUP98/RAP1GDS1 fusion gene.**

| *Features* | *NUP98/RAP1GDS1 Negative (No. 41)* | *NUP98/RAP1GDS1 Positive (no. 2)* |
|---|---|---|
| Sex | | |
| Male | 34 | – |
| Female | 7 | 2 |
| Age (years) | | |
| ≤ 30 | 31 | 1 |
| 31–50 | 8 | 1 |
| 51–60 | 2 | – |
| WBC count (×10⁹/L) | | |
| ≤ 5 | 18 | 1 |
| 0–100 | 14 | – |
| > 100 | 9 | 1 |
| Cytogenetics | | |
| Normal karyotype | 6 | 1* |
| t(4;11)(q22;p14) | – | 1 |
| t(1;19)(q23 ;p13) | 1 | – |
| Simple karyotype alteration | 7 | – |
| Complex karyotype alteration | 2 | – |
| No metaphases | 14 | – |
| Not done | 11 | – |

**t(4;11)-positive at relapse.*

*Key words: T-ALL, NUP98/RAP1GDS1 fusion gene, frequency.*

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