tern of fluorescence histogram with or without CSFs. Nevertheless, the histogram uniformly had a single peak or two narrowly split peaks. Accordingly, there exists a continuous, but not discrete, gradient of hTERT promoter activity among individual cells. To monitor the changes in hTERT promoter activity during short-term culture, hTERT-Venus-transduced AML cells were maintained with CSFs (Figure 2B). In UPN5, the viable cell fraction, determined using a FSC *vs*. SSC dot plot, decreased progressively, whereas the Venus expression in this fraction increased transiently at day 6 and declined at day 10. In UPN15, both the viable cell fraction and its Venus expression increased at day 6. The viable cell fraction decreased thereafter, but the fluorescence intensity of this fraction still increased at day 10. This might be due to the gradual disappearance of the cell fraction with lower hTERT promoter activity. An interesting issue is whether leukemia cells showing high hTERT promoter activity are included in the leukemia stem cell (LSC) compartment. If most LSCs exit from the cell cycle as do hematopoietic stem cells (HSC), hTERT promoter activity is likely to be repressed in LSCs, and would not be a related parameter.

In conclusion, we present a promising new method to monitor the hTERT promoter activity on a single living cell level. This method will help examine a relationship between hTERT promoter activity and replicative potential among primary leukemia cells.

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Key words: AML, telomerase, hTERT promoter, lentiviral vector.

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Expression of the nuclear oncogene Ski in patients with acute myeloid leukemia treated with all-trans retinoic acid

For acute myeloid leukemia (AML), a two-hit model has been proposed for leukemogenesis:[1,2] the first mutation involving tyrosine kinase signaling such as a RAS mutation or activation of this pathway via Flt-3-mutation, the second aberration via truncated or fusion transcripts caused by chromosomal translocations, deletions or inversions.[3]

We have recently found that the nuclear oncogene Ski is overexpressed in AML, especially in AML with deletion of chromosome 7.[4] Ski represses all-trans retinoic acid (ATRA) signaling and myeloid differentiation in AML cells *in vitro*. Expression of a Ski mutant unable to interact with N-CoR (nuclear receptor corepressor) and thus unable to co-operate with histone deacetylases (HDAC)[5] did not inhibit ATRA signaling. *In vitro* treatment of cells with the HDAC inhibitor valproic acid abrogated the inhibitory effect of wild type Ski.[4]

ATRA has proved to be a highly efficient agent in the therapy of patients with acute promyelocytic leukemia (APL; FAB classification M3) characterized by the fusion protein PML-RARα, which results in impaired retinoic acid signaling.[6] Discrepant results have been published regarding the use of ATRA in AML other than APL. A study from the British Medical Research Council (MRC) did not find a significant difference in survival of AML patients except AML-M3 up to the age of 55 years treated with chemotherapy versus chemotherapy plus ATRA.[7] By contrast, a study of the German-Austrian AMLSG study group including 242 AML patients >60 years, excluding APL patients, revealed a significant

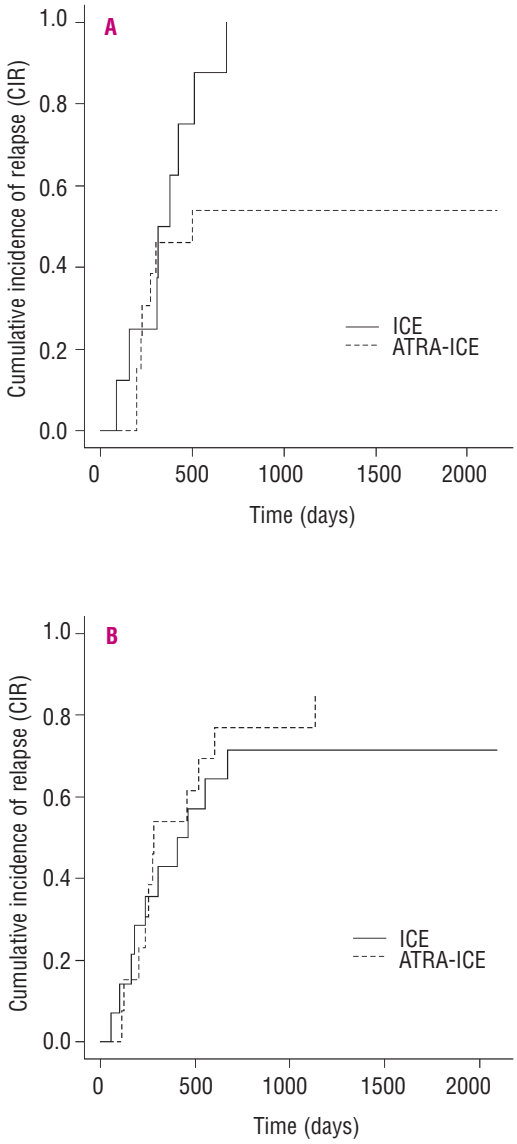

Figure 1. Cumulative incidence of relapse for the 48 elderly acute myeloid leukemia patients in complete remission treated with ICE versus ICE plus ATRA with low (A) or high (B) Ski expression level. The cut-off level was arbitrarily defined at the median Ski expression (low Ski ≤0.36; high Ski >0.36). Gray's k-sample test for (A) is p=0.11, for (B) p=0.57.

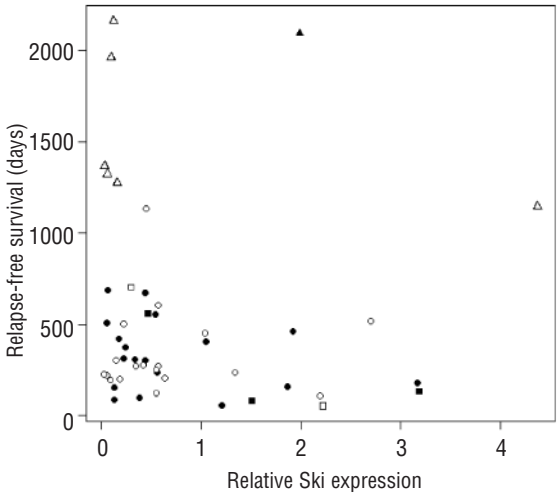

Figure 2. Relative expression level of Ski related to relapse free survival in the samples of the 48 elderly acute myeloid leukemia patients in complete remission. Open circles indicate ATRA-ICE treatment, events of relapse; closed circles indicate ICE treatment, events of relapse; open triangles indicate ATRA-ICE treatment, censored observations; closed triangles indicate ICE treatment, censored observations; open squares indicate ATRA-ICE treatment, cases of death due to other (competing) causes; closed squares indicate ICE treatment, cases of death due to other (competing) causes.

advantage for the group receiving ATRA together with ICE (idarubicin, cytarabine, etoposide) versus ICE alone. The combination improved complete remission (CR) rates, event free survival and overall survival (OS) in these AML patients. However, in this study the ATRA schedule and age were different from the MRC trial.[8]

The clinical data, showing a slight advantage for patients treated additionally with ATRA, together with our *in vitro* data, showing that Ski is a repressor of retinoic acid signaling, suggested the hypothesis that ATRA treatment should be more efficient in patients with low Ski expression compared with high Ski expression.

We retrospectively analyzed Ski expression in a subsample of 134 patients randomized into both arms of the AMLSG HD98B study[8] for whom leukemia samples were still available. In 2 out of 134 patients, cDNA synthesis was not sufficient. Out of the remaining 132 patients, 65 patients had received ICE (12 mg/m^2 idarubicin, day 1 and 3; 100 mg/m^2 cytarabine, days 1 to 5; 100 mg etoposide, day 1 and 3) as induction chemotherapy, and 67 patients were treated with chemotherapy ICE plus oral ATRA (45 mg/m^2; days 3 to 5 and 15 mg/m^2 days 6 to 28). Criteria for response and definition of relapse have been previously described.[8]

RNA extraction and cDNA synthesis were performed using standard protocols.[4] Quantitative Ski PCR was performed as previously described.[4] The staff of the molecular laboratory was blinded for the clinical data. Statistical analysis was carried out using a SPSS software package 12.1 as well as the software package R[9] (*http://www.R-project.org*). OS was calculated using the Kaplan-Meier method and cumulative incidence of relapse (CIR) was analyzed by a competing risk analysis. Differences between groups were evaluated using the log-rank test and Gray's k-sample test.[10] The median Ski expression was chosen as the arbitrary cut-off level. Accordingly, low Ski expression was defined as ≤0.36.

Statistical analysis showed that both groups of patients (ATRA-ICE versus ICE) were comparable for age, cytogenetic risk, lactate dehydrogenase level and leukocyte count. There was no difference in expression of Ski in patients randomized to ICE only or ICE plus ATRA. A comparison of patients whose AML cells expressed Ski at high levels (Ski >0.36) with AML patient cells with low Ski levels (Ski <0.36) found no difference in OS (p=0.57). Out of the 132 patients, 48 went into CR. Response to chemotherapy (CR, refractory disease and early death) was not associated with Ski expression level.

According to our hypothesis, ATRA should best protect patients with low Ski expression from relapse. Therefore, CIR and relapse free survival were evaluated in both treatment arms according to low and high Ski

expression. Analysis of CIR for patients treated with versus without ATRA (26 patients with ATRA-ICE *vs.* 22 patients with ICE) showed a distinct difference for the subgroup with high or low Ski expression (Figures 1A and 1B). These 48 patients were also analyzed for relapse free survival (RFS). There was a trend towards longer RFS in patients with low Ski expression randomized to ATRA therapy. Of 8 patients with RFS over 1,000 days, 7 were in the ATRA-ICE, and one in the ICE group. However, the Gray test for the low Ski group was not significant (p=0.11). Interestingly, the cells of the 5 long-term RFS patients randomized to ATRA had very low Ski levels compared with other AML patients (relative Ski expression of those 5 patients: 0.03; 0.059; 0.098; 0.12; 0.158; compared with range 0.023-4.39 in all 132 AML samples (median 0.36)). Of the 5 patients with longest RFS, 4 were in the low and one in the high Ski group (Figure 2). These data suggest that patients with very low Ski expression levels might be a special subgroup responsive to ATRA treatment.

To summarize, there was a trend to prolonged RFS for patients in CR with low Ski expression treated with additional ATRA compared with those who received ICE treatment only. Interestingly, long term survivors (>1,000 days) treated with ATRA had very low Ski expression levels. In AML, low Ski expression seems to mark a subgroup responsive to ATRA when given in combination with ICE during induction chemotherapy. This should be addressed in future trials.

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NOTCH2 mutations in marginal zone lymphoma

The Notch family of proteins are highly conserved cell surface receptor proteins that are important for cell fate decisions and cell differentiation of various tissues of the body.[1] Ligand activation initiates a cascade of proteolytic events that translocates the intracellular part of the Notch protein to the nucleus where it activates different target genes. Some main targets are known.[1] However, much of the intricacies of the Notch signaling pathway still need to be discovered. It has been known for a while that Notch1 has a critical role in T versus B cell fate development from a common progenitor in the thymus[2-4] and is involved in leukemogenesis. Activating NOTCH1 mutations were recently found in more than 50% of T-ALL patients.[5] The mutations affect two main regions, the extracellular heterodimerization domain (HD) and the C-terminal transcriptional activation domain (TAD) or PEST domain.[5] Notch2 on the other hand promotes the selective development of B1 B lymphocytes[6] and is predominantly expressed in mature B cells.[7] Furthermore, several studies have shown that Notch2 is indispensable for the development of marginal zone B cells.[8-10] As a first step to investigate the involvement of Notch2 in B-cell lymphoma development, we studied oncogenic NOTCH2 mutations in several types of B-cell malignancies. The samples studied comprised 41 cases of marginal zone lymphoma (MZL) and 28 cases of other B-cell lymphoma types. The MZL were diagnosed as splenic (28 cases), nodal (3 cases) or extranodal MALT-type (10 cases) and were obtained from the Department of Pathology, The Norwegian Radium Hospital and from the Department of Pathology, University Hospital of Leuven, Belgium. All other lymphoma types were obtained from the Department of Pathology, The Norwegian Radium Hospital. In a selection of the lymphoma patients, tissues not involved by lymphoma were also studied. Additionally, two marginal zone cell lines, SSK41 and Karpas 1718, were studied. The procedures followed in the present study were in accordance with institutional ethical standards.

The detection of potentially oncogenic mutations in