not need an alloSCT for cure after relapse. Results of the ongoing International BFM Study Group Pediatric Relapsed AML protocol, will contribute to this discussion in the near future. The Dutch and literature data suggest that randomized studies of the role of alloSCT in subgroups of relapsed AML are required. Meanwhile, experimental allogeneic SCT procedures, such as haplo-identical and mismatched unrelated donor SCT, should be carefully balanced against the chance of cure by chemotherapy only in patients in good quality second complete remission of AML.

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The International Prognostic Scoring System for Waldenström's macroglobulinemia is applicable in patients treated with rituximab-based regimens

Waldenström's macroglobulinemia (WM) is characterized by lymphoplasmacytic bone marrow infiltration and by production of serum monoclonal IgM.[1] This disease usually follows a relatively indolent course with a median survival ranging from 60 months to 120 months in different series. However, in some patients the disease may be more aggressive causing their death within months.[1-7] Several analyses have identified variables that may be associated with a worse prognosis. These include advanced age, cytopenia(s), hypoalbuminemia, elevated serum β2-microglobulin, high IgM, poor performance status, B-symptoms or splenomegaly.[1-8]

Recently, a multicenter collaborative project was undertaken which included a large number of previously untreated, symptomatic patients who required treatment. An International Prognostic Scoring System for WM (IPSSWM) was formulated based on 5 adverse covariates: age >65 years, hemoglobin ≥11.5 g/dL, platelet count ≤100×10^9/L, β2-microglobulin >3 mg/L and serum monoclonal protein concentration >70 g/L. Low risk is defined by the presence of ≤1 adverse variable except age, high risk by the presence of >2 adverse characteristics and intermediate risk by the presence of 2 adverse characteristics or age >65 years; 5-year survival rates are 87%, 68% and 36% respectively.[9]

However, 96% of the patients included in this analysis had received frontline treatment with alkylating agents or nucleoside analogs. Since rituximab-based regimens are now frequently used as a frontline treatment in WM patients, we analyzed whether the IPSSWM can be applied in these patients.

Ninety-three previously untreated, symptomatic patients who received treatment either with single agent rituximab (21 patients) or with the combination of dexamethasone, rituximab and cyclophosphamide (72 patients), formed the basis of this analysis. This analysis was approved by the Institutional Review Board. These patients were included in two trials reported previous-

ly.[10,11] Briefly, in a single agent rituximab study, rituximab was administered at a dose of 375 mg/m^2 IV weekly for four consecutive weeks. Three months after completion of rituximab, patients without evidence of progressive disease received repeat 4-week courses of this agent.[10] The DRC regimen consisted of dexamethasone 20 mg followed by rituximab 375 mg/m^2 IV on day 1. Oral cyclophosphamide 100 mg/m^2 BID was administered on days 1 to 5. DRC courses were repeated every 21 days for 6 courses.[11] Eighty percent of non-responding patients in either trial were treated with either fludarabine-based regimens (i.e. with cyclophosphamide or with mitoxantrone) and the remaining patients received chlorambucil. The disease features of the 93 patients were typical of symptomatic WM (Table 1). There was no difference in the characteristics of patients treated either with single agent rituximab or with DRC, except that B-symptoms were more common in the DRC group. Criteria for initiation of treatment included cytopenia(s) (in 48% of patients), hyperviscosity (24%), constitutional symptoms (9%), organomegaly or lymphadenopathy (8%), IgM related disorders (6%) and miscellaneous reasons (5%).

According to the IPSSWM, 15% of patients were classified as low risk, 65% as intermediate risk and 20% as high risk. Median follow-up for all patients was 30 months. Overall survival for all patients has not been reached and 70% of patients were alive at five years. The median follow-up time for the single agent rituximab-treated patients is 72 months and for the DRC patients 23.4 months. There was no significant difference in median cause-specific survival between the two treatment groups (p=0.150). Among patients of the three different risk groups, there was a significant difference in overall survival: median survival has not been reached for low or intermediate risk group and is 38 months for high risk patients (p=0.006) (Figure 1A). The 4-year survival probability for all patients was 100% for low risk, 73% for intermediate risk and only 41% for high risk patients. However, we should mention that the follow-up of our patients is relatively short and this may result in inherent inaccuracies because of the high number of censored observations. At the time of last follow-up, 19 patients had died and 100%, 82% and 58% of patients were alive in the low, intermediate and high risk groups respectively. Among the 19 deaths, 5 (26%) were considered not to be related to WM or to treatment complications. Thus, 14 deaths were included in the construction of cause-specific survival curves, with patients dying of unrelated causes censored at the time of their death. There was a significant difference between the three cause-specific survival curves (p=0.05) (Figure 1B).

Treatment options for WM include alkylating agents, nucleoside analogs, monoclonal antibodies alone or in combination, and intensive therapy with stem cell support.[1,12] Since some of these treatments may be associated with significant toxicity, a reliable prognostic scoring system may help to select the patients more likely to benefit. In our current analysis, we showed that the IPSSWM is also applicable in patients who received primary treatment with rituximab-based regimens. Furthermore, we were able to show that the IPSSWM is also valid for cause-specific survival. This finding is important, since unrelated causes lead to the death of a significant proportion of WM patients (one fourth in this series) thus biasing survival calculations.[6]

The use of IPSSWM can also identify subsets of patients who are less likely to benefit from current treat-

Table 1. Patients' and disease features.

	All patients (%)	*Single-agent Rituximab (%)*	*DRC (%)*	*p value*
Age>65 years	63	69	61	0.470
Males	65	73	61	0.282
B-symptoms	22	8	27	0.032
Splenomegaly	29	27	30	0.830
Lymphadenopathy	28	31	36	0.282
Hemoglobin ≤11.5 gr/dL	77	70	73	0.880
Platelets ≤100×10^9/L	13	19	10	0.263
Serum peak >70 gr/L	8	15	5	0.086
B2-microglobulin>3 mg/dL	71	58	64	0.292
IPSS				
Low	15	19	13	
Intermediate	65	54	69	0.407
High	20	27	18	
Cause-specific survival (months)	Not reached	Not reached	39	0.150

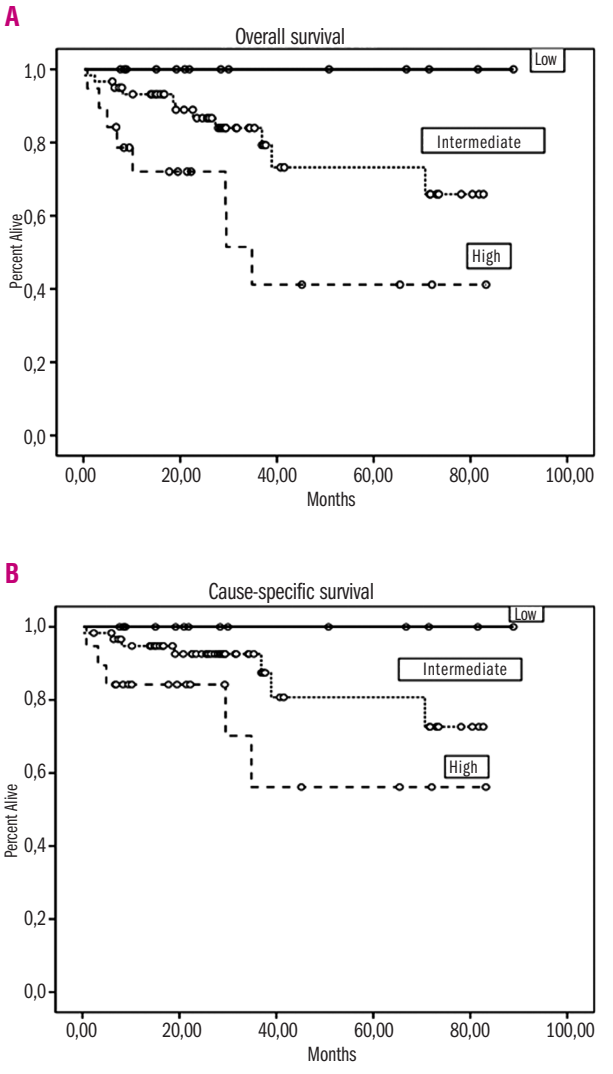

Figure 1. Overall (1A) survival and cause-specific (1B) survival for the different IPSSWM groups.

ment options. We observed that those of our patients treated with rituximab-based regimens who were assigned to the high risk group had a median overall and cause-specific survival of less than four years. When such patients were treated with alkylating agents or with nucleoside analogs median overall survival was also less than four years.[9] This observation indicates that rituximab-based regimens, as well as nucleoside analog/alkylating agent based regimens, may be a suboptimal treatment for such high risk patients. For these patients new treatment approaches are needed.[12]

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ZAP-70 mRNA expression provides clinically valuable information in early-stage chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is a heterogeneous disease with a variable clinical course and overall survival times ranging from months to decades.[1] Mutational status of immunoglobulin heavy chain variable region (IGHV) correlates with clinical behavior and is a powerful prognostic factor in CLL. Unmutated IGHV patients have a reduced survival and poor responsiveness to chemotherapy,[2,3] however, IGHV sequencing is difficult to perform in a routine diagnostics laboratory. Gene profiling studies indicated that *ZAP-70* was the gene that best distinguished between IGHV groups[4] and that it could serve as an independent prognostic factor that is expressed in a stable manner during the course of the disease.[5-7] On the other hand, assessment of *ZAP-70* by flow cytometry (FC) presents some technical difficulties since T and NK cells express *ZAP-70* and must be excluded from the analysis.

Our objective was to evaluate the prognostic significance of *ZAP-70* determined by real-time PCR (RTqPCR) in early-stage CLL patients and compare its performance with FC analysis and IGHV mutational status to identify patients at risk of progression.

We studied 70 samples from untreated CLL patients (Binet stage A) after obtaining their informed consent. Rearranged *IGHV* genes were amplified by PCR using a standard protocol.[8] We considered unmutated those samples with >98% homology with the closest germinal line.

FC analysis of *ZAP-70* was performed on fresh samples (n= 69) according to Crespo *et al.*[6] with some modifications. An isotype control was used as negative control. Results ≥20% were considered positive.

For RTqPCR assays, RNA was prepared from 7-10×10^6 $CD19^+$ selected cells and amplification was carried out using Hs00277148_m1 primers and probe sets (TaqMan® Gene Expression Assays, Applied Biosystems). Amplification of GUS gene was performed in all cases to normalize gene expression.

Time to progression (TTP) was calculated from the date of diagnosis to the date of disease progression (based on NCI guidelines) or last follow-up. All statistical calculations were performed using the SPSS 13.0 software. Out of the 70 patients studied, 20 (28.6%) were unmutated. The most common families used in mutated samples were $V_{H3\text{-}7}$ (10.0%), $V_{H2\text{-}5}$ (10.0%), $V_{H3\text{-}30}$ (8.0%), and $V_{H1\text{-}3}$ (8.0%), whereas unmutated patients were $V_{H1\text{-}69}$ (30%) and $V_{H3\text{-}33}$ (15%).

The mean ± 2 SEM values of *ZAP-70* measured by