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A progression-risk score to predict treatment free survival for early stage chronic lymphocytic leukemia patients

Running title: Progression-risk score for CLL

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Several phenotypic, molecular, and chromosomal markers of chronic lymphocytic leukemia (CLL) cells have been identified that are significantly associated with patient prognosis (1-6). However, these markers used singularly are inaccurate predictors of outcome for individual patients. Recent efforts have focused on combining markers to predict either treatment-free survival (TFS) (4,7,8) or overall survival (OS) (9-11), however further effort is worthwhile to determine how to combine prognostic parameters, optimize risk stratification, simplify calculations, and/or identify new prognostic variables.

Herein, analyzing data from a cohort of Binet A patients, enrolled in a prospective multicenter observational study, we developed a weighted, multivariate score [progression-risk score (PRS)] integrating clinical, laboratory and biological parameters independently associated with TFS. The PRS was subsequently validated using an external cohort of CLL patients from the Mayo Clinic, Minnesota, USA.

We analyzed data from 480 newly diagnosed CLL patients enrolled in the O-CLL1-GISL protocol (clinicaltrial.gov identifier: NCT00917540). Of these, 337 cases with available biological (CD38, ZAP-70, *IGHV* mutational status, and FISH) and clinical/laboratory parameters [(sex, age, absolute lymphocyte count (ALC), Rai modified stage, lactate dehydrogenase (LDH; normal range, 313-618 IU/L) and β2-microglobulin level (normal range, 0.6-2.0 mg/L)] (11), were included in this analysis (see Supplementary Methods).

Factors independently associated with TFS were included in the PRS. To account for differences in the magnitude of the association between individual independent factors and TFS, we assigned a weighted-risk score to each factor based on ranges of their corresponding hazard ratios (HR) (i.e., 1 point for HR 1.1-1.9; 2 points for HR 2.0-2.9, etc.) (9). The total risk score was then calculated by summing the ratings of each individual factor. Risk groups were identified combining risk categories with a non-statistically different TFS (see Supplementary Methods).

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Baseline patient features of the training cohort are listed in Supplementary Table 1. Patients with Rai stage I and II were grouped for analysis according to convention (12). Given the limited number of patients with del(11q23) and del(17p13), cytogenetic abnormalities identified by FISH were clustered in 3 risk groups (i.e. low-risk [del(13q14) and normal], intermediate-risk [trisomy 12], and high-risk [del(11q23) and del(17p13)]). After a median 42-month follow-up (range, 6–82 months), 84/337 (24.9%) cases required treatment.

In multivariate analysis, Rai stage I–II, ALC≥10x10^9/L, elevated β2-microglobulin levels, and *IGHV-UM* remained associated with shorter TFS (Table 1). The multivariate model was confirmed by bootstrap resampling (data not shown). Considering the HR of the independent factors, a risk score was assigned to each marker (Table 1); the total risk score was defined as the sum of the risk scores of the four individual parameters (range, 0–7). According to the predefined criteria (Supplementary Table 2), three different risk categories for TFS were determined: low- (score 0–2), intermediate- (score 3–5), and high-risk (score 6–7) (Supplementary Table 3).

According to the PRS in the training cohort 178 patients (52.8%) were classified as low-risk, 126 (37.4%) as intermediate-risk, and 33 (9.8%) as high-risk (Supplementary Table 4). Low-risk, intermediate-risk and high-risk patients had significantly different TFS (Figure 1A and Supplementary Table 4). The C-statistic was 0.75 (P<0.001) for predicting TFS. The score appeared well-calibrated since the Hosmer-May test was not significant ($\chi 2$=0.82; P=0.36), indicating that predicted and observed risks were very close.

The validity of the score was evaluated in a cohort of 428 early stage CLL patients prospectively diagnosed and followed-up at the Mayo Clinic. Baseline patient features of the validation set are listed in Supplementary Table 5. For β2-microglobulin, the first result obtained at the Mayo Clinic laboratory (all within 18 months of diagnosis), was used for analysis. At last follow-up, 298/428 (69.6%) cases remained untreated (median follow-up=97 months, 95%CI: 82-113 months). According to the PRS, 174 cases (40.6%) were at low-risk, 178 cases (41.6%) at intermediate-risk,

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and 76 (17.8%) at high-risk for disease progression. Low-risk, intermediate-risk and high-risk patients had significantly different TFS (Figure 1B and Supplementary Table 3). The C-statistic was 0.72 (P<0.001) for predicting TFS; the PRS again appeared well-calibrated, as the Hosmer-May test was not significant ($\chi 2$=0.65; P=0.72).

Subsequently, we compared the PRS to the MDACC model (12) by calculating total point scores according to their proposed formula in both training and validation cohorts (see Supplementary Methods). The total point scores in the training cohort ranged from 0 to 74.5 points (median=19). The C-statistic of the MDACC model was 0.69 (P<0.001), slightly below our score (training cohort=0.75). The total point scores of the validation cohort ranged from 0-84.9 points (median=12). The C-statistic of the MDACC model in the validation cohort was 0.71 (P<0.001), similar to our score (validation cohort=0.72). Moreover, the Akaike information criterion [(AIC); lower score more favorable)] indicated that in both the training (PRS, AIC=795.312 versus MDACC model, AIC=839.561) and validation cohorts (PRS, AIC=1287.52 versus MDACC model, AIC=1324.27) that the PRS was superior to the MDACC model for predicting TFS. Calculating Akaike weigths, in the training cohort the PRS had a 99% chance of being the best model compared to the MDACC model (1%), which was also true in the validation cohort (PRS: 99%; MDACC model: 1%). The explained variation in the incidence rate of the study outcome (i.e. an index combining calibration and discrimination in the setting of Cox regression analysis) was attributable to the PRS (training cohort: 38%: validation cohort: 30%) and the MDACC model (training cohort: 19%; validation cohort: 17%) reasonably indicated that the PRS had a consistently higher prognostic accuracy compared to the MDACC model for predicting TFS.

Our PRS allows stratification of early stage patients in terms of TFS. This endpoint has some advantages over prognostic tools designed to predict OS for CLL patients in early stages, since it is a disease specific end-point that is not limited by competing risks of death due to unrelated health conditions and is not limited by the impact of new therapies on OS. The validity of PRS was also

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validated in an independent cohort. Furthermore, although PRS is based on a lower number of parameters and a more simplified calculation, it appears to more accurately predict TFS than the MDACC model (11).

Some aspects in our study should be critically evaluated. First, patients >70 years, representing nearly half of the CLL cases, were excluded from our training cohort based on our O-CLL-1 protocol criteria. Nonetheless, the sub-analyses performed in the validation set demonstrated that the PRS score predicted TFS also when patients were clustered into two subgroups according to age (i.e. $\leq$70 and >70 years) (Supplementary Figure 1A and B). Second, ZAP-70 expression and FISH results were not independently associated with TFS in our cohort. The lack of standardized methodology for evaluating ZAP-70 expression, the limited number of progression events, and the low number of cases with del(17p13) and del(11q23) might contribute to the lack of significance of these parameters. Third, since this observational trial started in 2007 and CLL diagnosis and staging were based on the NCI-WG 1996 guidelines (13), a significant fraction of cases (107/337; 31.7%) included in our cohort would be reclassified as clinical monoclonal B-cell lymphocytosis (cMBL) by IWCLL 2008 guidelines (14). Excluding these 107 cMBLs, the remaining 230 cases when classified according to PRS showed a significantly different TFS (Supplementary Figure 2). Fourth, newer biological markers have not yet been evaluated in our model. Among these is CD49d expression, a powerful flow cytometry-based prognostic marker (5), which was not evaluated in our cohort of patients. Furthermore, *NOTCH1* and *SF3B1* gene mutations (10) were not included in this analysis. Although we have characterized *SF3B1* mutational status in 170 and *NOTCH1* in 270 of these patients, neither showed any independent predictive value on TFS (data not shown). Finally, there currently are not enough events in our cohort to evaluate the association of our risk score with OS.

The GCLLSG, employing a similar statistical approach, recently developed a prognostic index based on clinical and biological parameters to predict OS with a C-statistic score exceeding 0.8 in

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the validation cohort (9). A recent international collaboration is also engaged in developing a comprehensive tool to predict OS (CLL-IPI score) (15). These tools have also been shown to predict TFS, although this was not the primary purpose for the score. The inclusion of a parameter such as the s-TK in the German index may restrict its use in clinical practice. The CLL-IPI score, based on 5 largely diffuse parameters (age, stage, del17p/*TP53* mutation, *IGHV* mutation status, and β2-microglobulin) could represent a simplified "globally applied" model, easily applicable in daily clinical practice, which allows to predict clinical course of CLL patients across all stages.

Overall, we believe that our study has several strengths. First, it is based on data from a multicenter cohort of newly diagnosed patients enrolled prospectively and well-characterized at centralized laboratories for major genetic abnormalities, cellular, and molecular markers. The PRS is easily applied, allows accurate stratification of early stage patients, and identifies those with an aggressive clinical course who may be candidates for clinical trials evaluating early treatment with novel effective therapies. Nevertheless, this PRS requires further validation in other independent cohorts with larger numbers of older patients.

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Figure legends

Figure 1 TFS according to progression-risk score. TFS according to progression-risk score (PRS) in the training set (A) and in the validation set (B).

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Table 1. Univariate and multivariate Cox proportional Hazards Models for TFS

Variable	Univariate analysis		Multivariate analysis		
	HR (95% CI)	***P***	**HR (95% CI)**	***P***	**score**
Age (years) <60/≥60	1.12 (0.73-1.74)	0.59	-	-	-
Sex Male/Female	0.93 (0.6-1.44)	0.93	-	-	-
Rai stage 0/I-II	2.30 (1.47-3.50)	<0.0001	1.76 (1.11-2.78)	0.015	0/1
ALC (10^9/L) <10/≧10	3.43 (1.99-5.92)	<0.0001	2.70 (1.54-4.72)	0.001	0/2
β-2 microglobulin normal/elevated	3.04 (1.96-4.70)	<0.0001	2.65 (1.66-4.21)	<0.0001	0/2
LDH normal/elevated	1.25 (0.57-2.71)	0.57	-	-	-
CD38 negative/positive	3.22 (2.06-5.02)	<0.0001	1.40 (0.80-2.42)	0.24	-
ZAP-70 negative/positive	2.34 (1.51-3.61)	<0.0001	1.0 (0.98-1.01)	0.72	-
IGHV mutated/unmutated	3.57 (2.32-5.50)	<0.0001	2.39 (1.27-4.50)	0.007	0/2
FISH risk low+int/high	2.93 (1.46-5.90)	0.002	1.80 (0.84-3.88)	0.13	-

Abbreviations: ALC: absolute lymphocyte count; CI: confidence interval; HR: hazard ratio.

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No. of pts at risk							
Low risk	178	167	146	123	76	43	10
Int risk	126	117	95	72	46	23	4
High risk	33	20	13	5	4	3	0

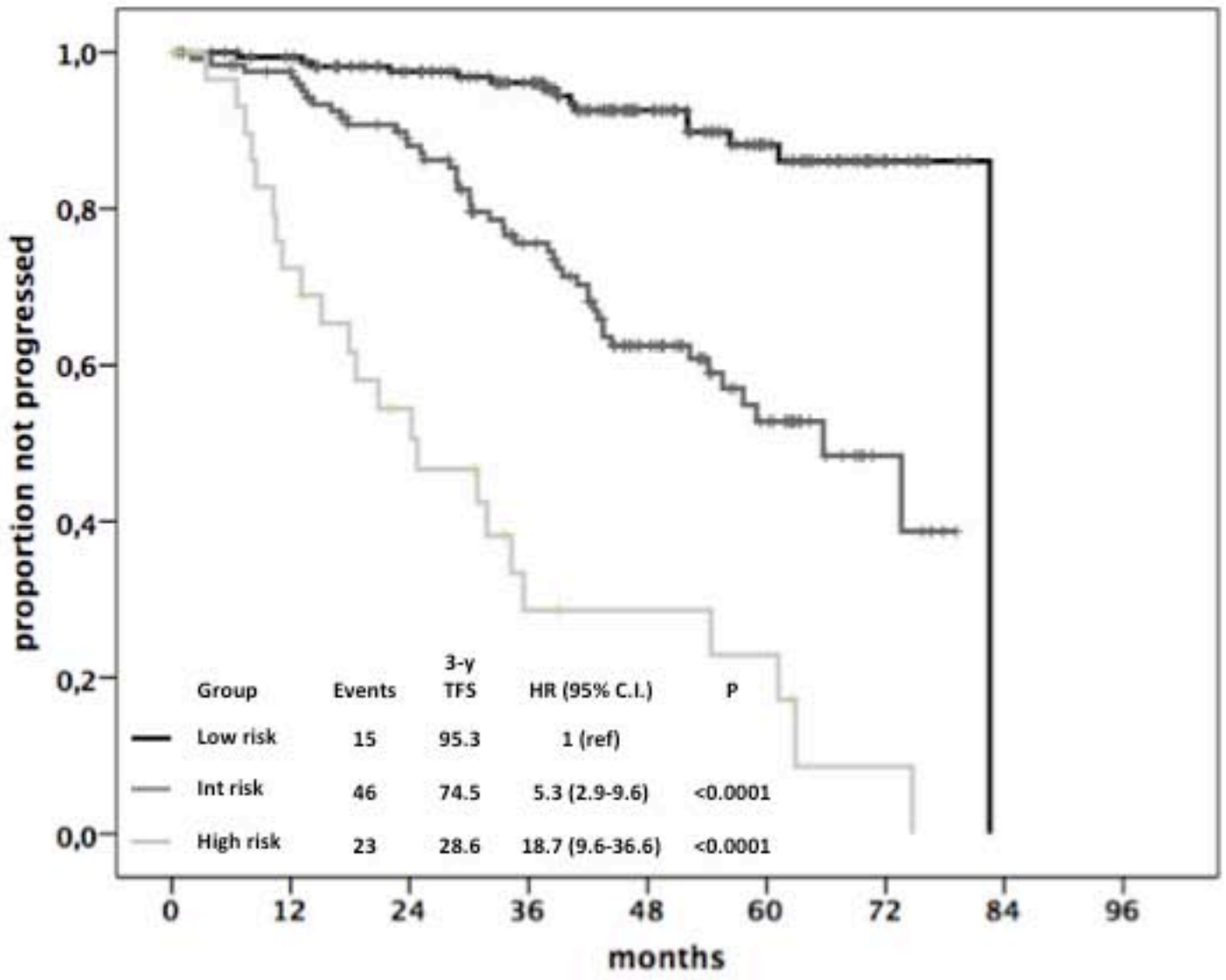

Figure 1A

No. of pts at risk												
Low risk	174	139	130	119	104	90	75	54	30	20	3	1
Int risk	178	127	112	94	81	58	43	30	15	4	1	0
High risk	76	34	21	16	13	9	2	1	1	0	0	0

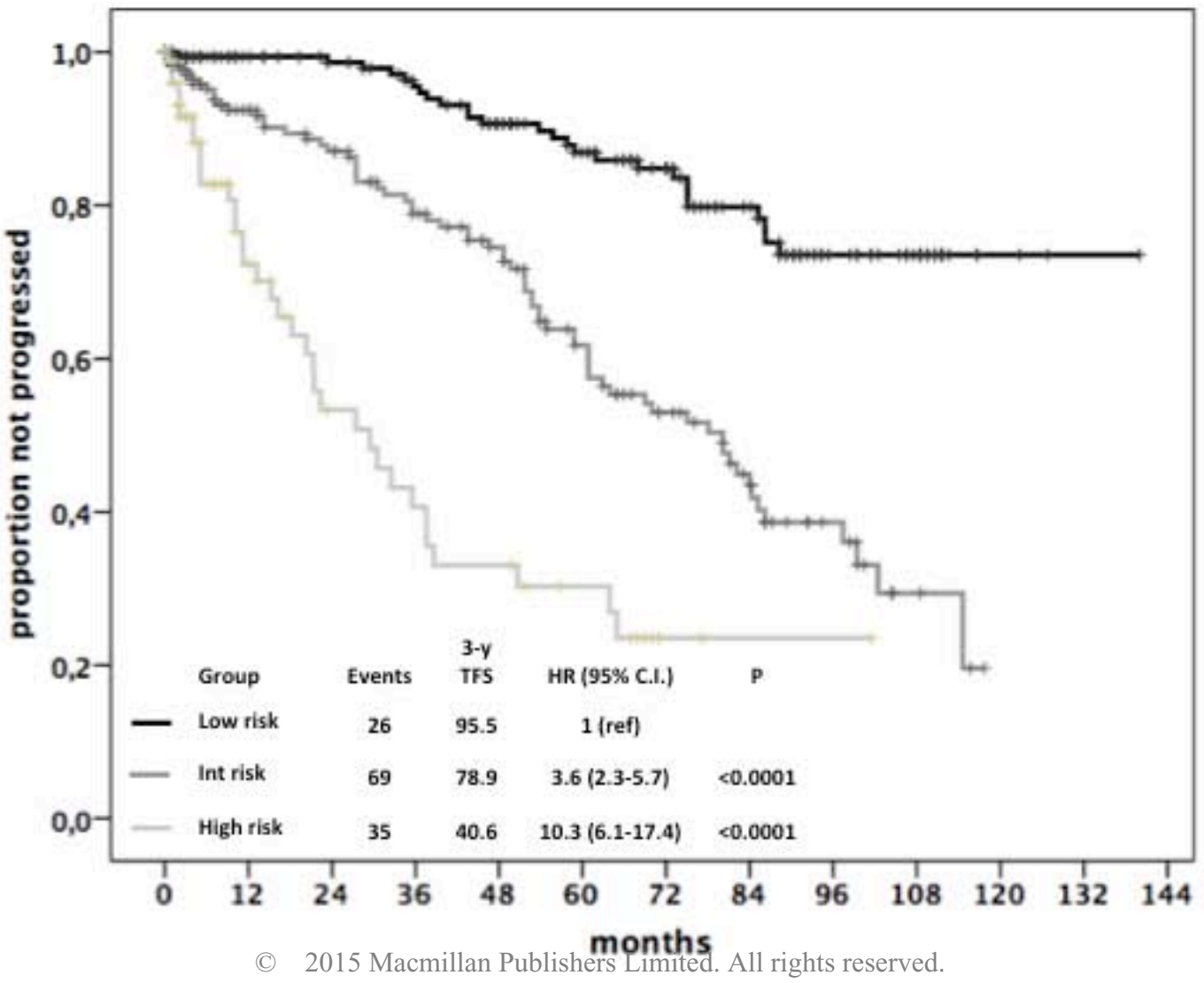

Figure 1B

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