A laboratory-based scoring system predicts early treatment in Rai 0 chronic lymphocytic leukemia

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Supplemental Materials for

A Laboratory-Based Scoring System Predicts Early Treatment in Rai 0 Chronic Lymphocytic Leukemia

Supplemental information:

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Supplemental Table 1. Clinical and laboratory features of CLL patients.

Parameter	n/total cases (%)				
	Training	**Gemelli**	**Cardiff**	**Mayo**	**O-CLL**
Age ≥65, years	254/478 (53.1)	92/144 (63.9)	225/395 (60.0)	241/540 (44.6)	97/322 (30.1)
WBC count >32x10^3 cell/μL	141/478 (29.5)	5/144 (3.5)	110/395 (27.8)	92/540 (17.0)	29/322 (9.0)
IGHV unmutated	94/478 (19.7)	35/144 (25.3)	83/395 (21.0)	178/540 (33.0)	97/322 (30.1)
FISH category					
del17p	30/478 (6.3)	3/144 (2.1)	17/395 (4.3)	18/540 (3.3)	6/322 (1.9)
del11q	36/478 (7.5)	6/144 (4.2)	32/395 (8.1)	38/540 (7.0)	18/322 (5.6)
tri12	67/478 (14.0)	14/144 (9.7)	39/395 (9.9)	59/540 (10.9)	38/322 (11.8)
Beta-2-microglobulin >3.5mg/L	36/418 (8.6)	21/144 (14.6)	92/395 (23.3)	70/540 (13.0)	1/239 (0.4)
Follow-up median, months (95%CI)	62 (57-68)	91 (83-103)	94 (83-104)	77 (68-88)	89 (85-95)

FISH categories were as reported by Dohner et al (Dohner H, N Engl J Med. 2000;343:1910-6). Abbreviations: WBC, white blood cell.

Supplemental Table 2. Treatment free survival in CLL cases identified as high-risk according to the CRO model, stratified by score (N=309).

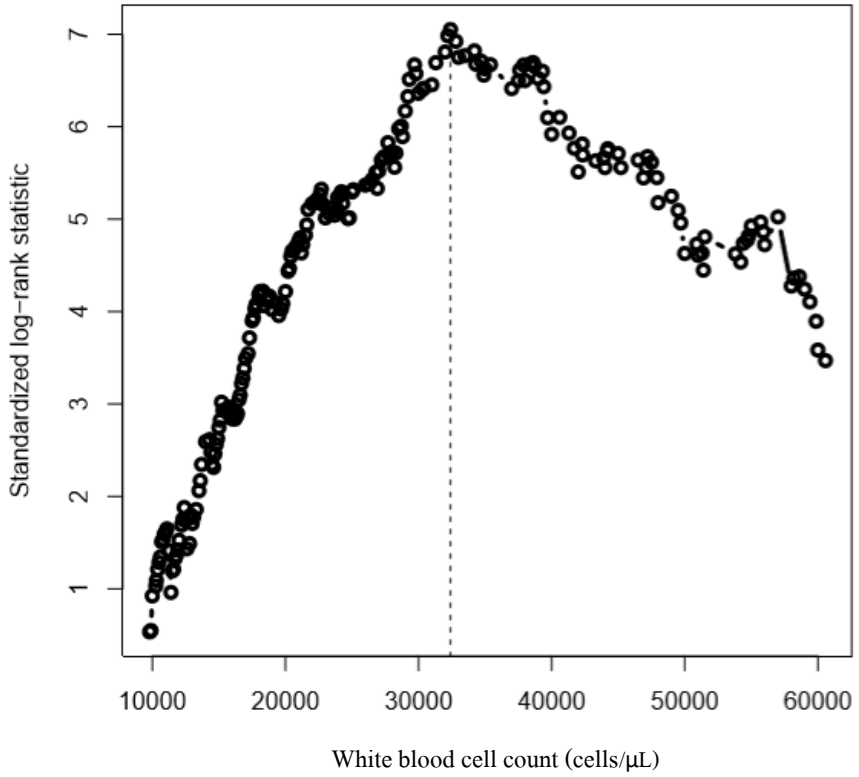
CRO score	Median, months	95% CI, months
3	57.6	45.9-68.0
4	44.0	36.0-64.0
5	28.0	14.7-29.1
Overall	51.6	42.6-60.0

CLL cases were taken from the training and validation cohorts. Abbreviations: CI, confidence interval.

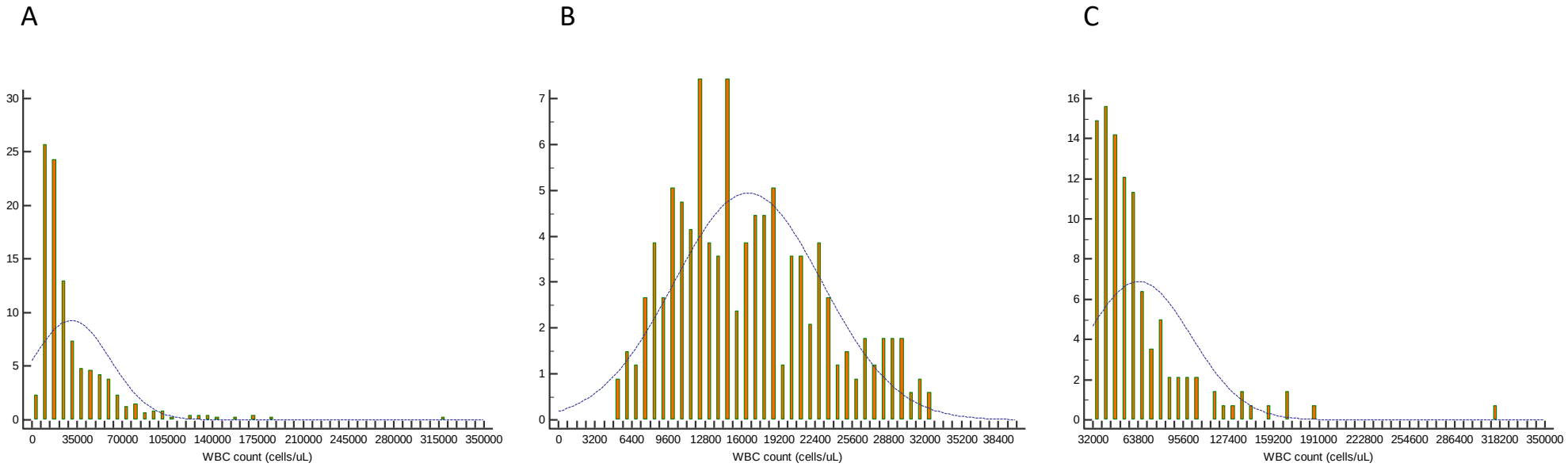
Supplemental Table 3: Subgroup univariable and reduced multivariable analysis involving 304/478 cases from the training cohort with available TP53 and NOTCH1 gene mutational status.

Factor	**Univariable Analysis**			**Reduced Multivariable Analysis**		
	P	**HR**	**95% CI**	**P**	**HR**	**95% CI**
WBC>32K cells/μL	<0.0001	2.69	1.80-4.00	0.0009	2.02	1.33-3.06
Dohner category						
del11q	<0.0001	4.25	2.41-7.49	0.0234	1.95	1.09-3.47
tri12	0.0001	2.75	1.67-4.53	0.0130	1.94	1.15-3.28
UM IGHV	<0.0001	3.98	2.64-5.98	<0.0001	2.57	1.64-4.04
TP53 disrupted	<0.0001	3.12	1.97-4.94	0.0086	2.01	1.94-3.39
Del17p	<0.0001	4.00	2.12-7.55			
TP53 mutated	<0.0001	3.33	2.07-5.38			
CD49d+	0.0022	1.85	1.25-2.75			
NOTCH1 mutated	0.0003	2.25	1.45-3.48			
Age >65 years	0.0818	1.42	0.96-2.12			
Male	0.9251	1.02	0.69-1.51			

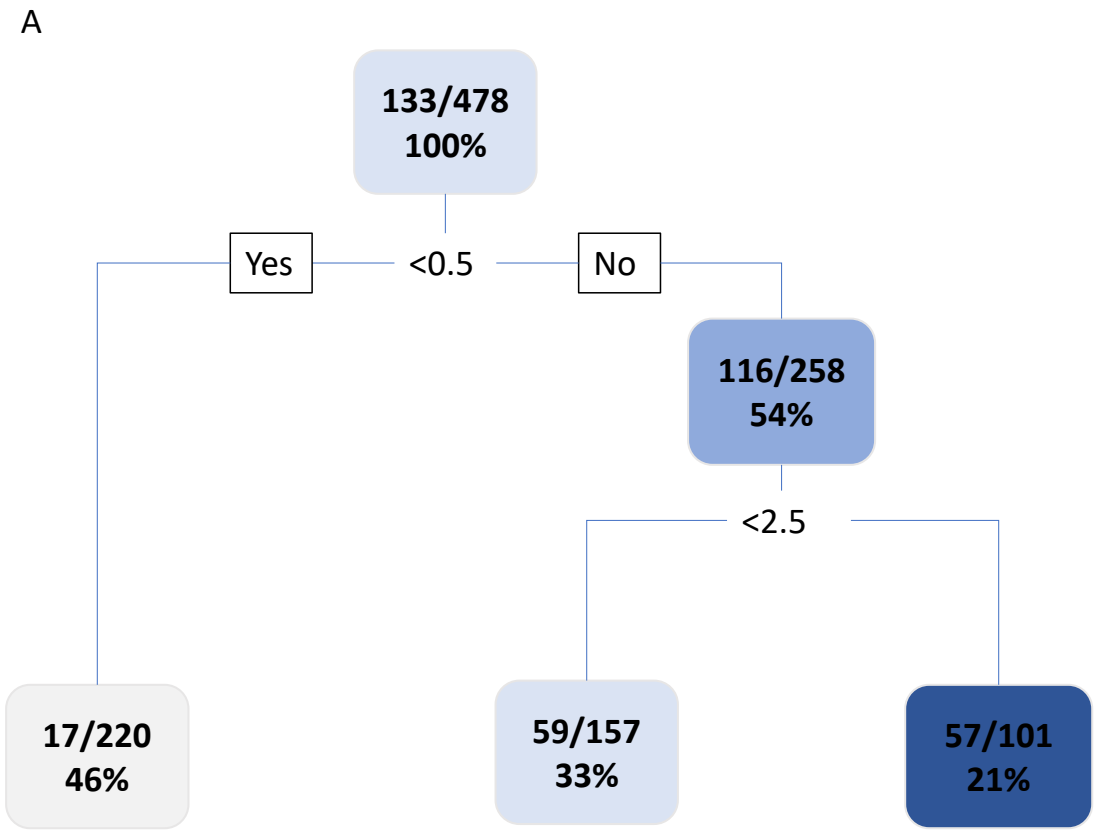
FISH categories were as reported by Dohner et al (ref. 7). Abbreviations: HR, hazard ratio; CI, confidence interval; WBC, white blood cell; UM, unmutated.



Supplementary Figure 1. Establishing a WBC count cutoff. Maximally selected log-rank statistic establishing a WBC count >32K cells/μL as our threshold.



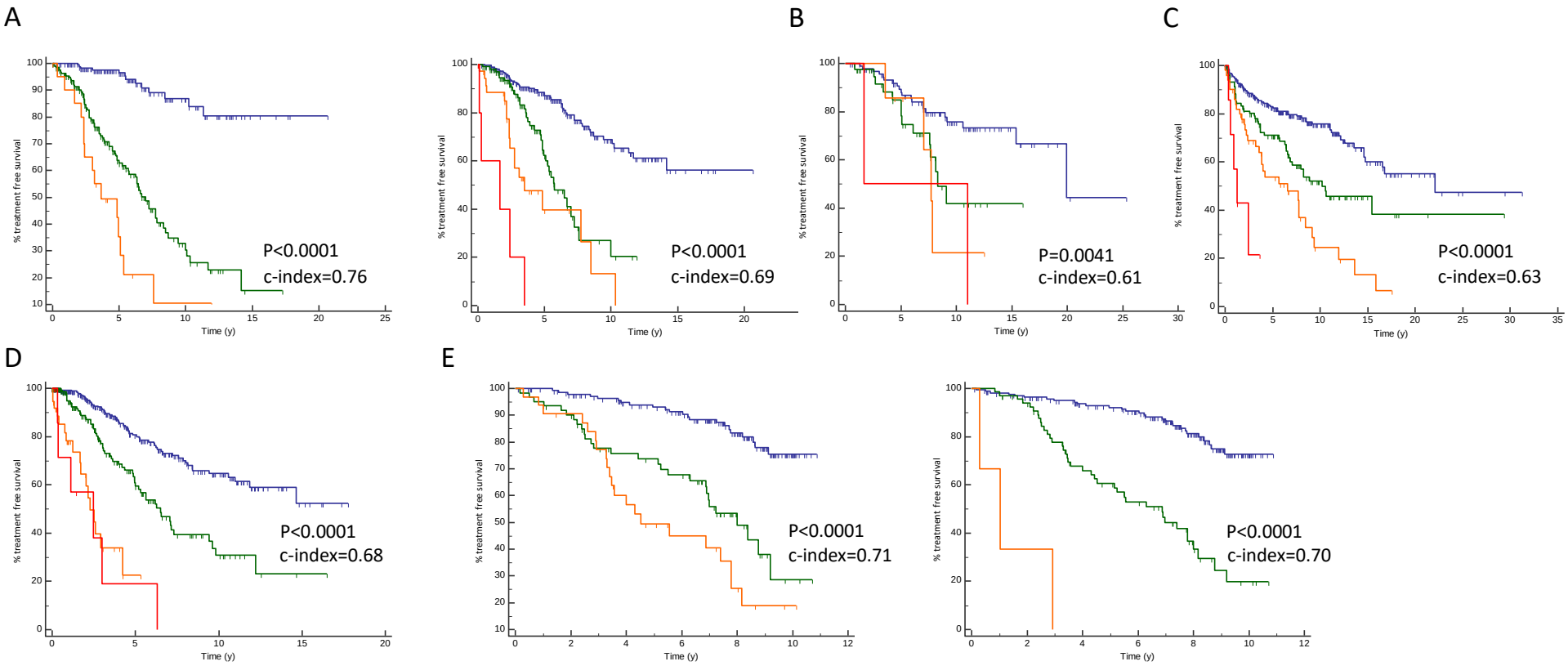
Supplementary Figure 2. Distribution plots demonstrating WBC count frequencies in the training cohort. Histogram of WBC count frequencies in (A) all 478 cases comprising the training cohort (B) cases with WBC count ≤32x10^3 and (C) cases with WBC count >32x10^3 cell/µL.



B

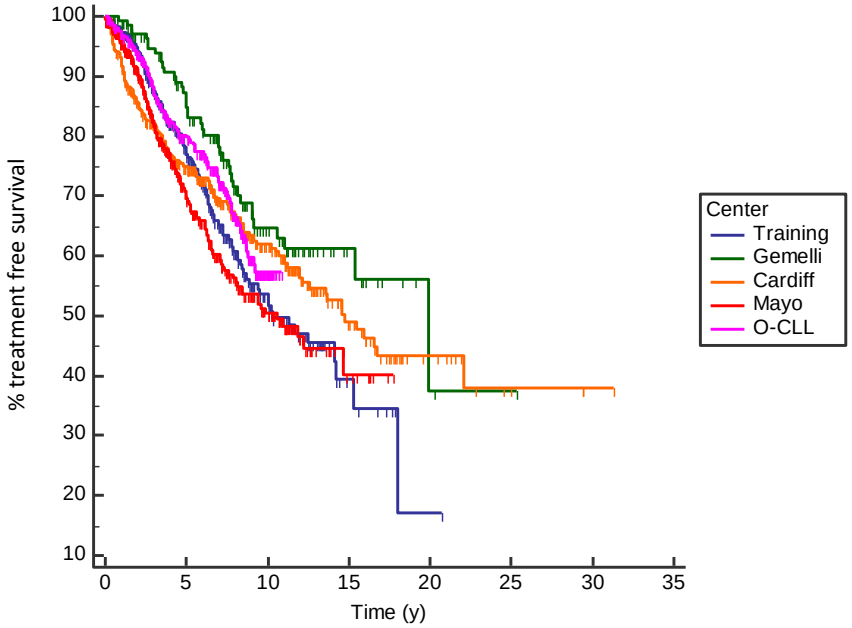
Score	Risk Group
0	Low
1-2	Intermediate
3-5	High

Supplementary Figure 3. Risk group determination. A) Recursive partitioning demonstrating 2 cut-offs at <0.5 and <2.5 (within nodes, # of events/cases and % of total cases reported). B) Risk group categorization into low (0) intermediate (1-2) and high (3-5).



Supplementary Figure 4. Comparison to CLL-IPI. Kaplan-Meier curves demonstrating treatment free survival (%, Y-axis) in: (A) the training cohort (left panel, CRO score; right panel, CLL-IPI); (B) the Gemelli validation cohort (CLL-IPI; the corresponding CRO score is reported in Figure 3A); (C) the Cardiff cohort (CLL-IPI; the corresponding CRO score is reported in Figure 3B); (D) the Mayo cohort (CLL-IPI) the corresponding CRO score is reported in Figure 3C); (E) the O-CLL cohort (left panel, CRO score; right panel, CLL-IPI) . Blue: low-risk category, green: moderate-risk category, orange: high-risk category, red: very high-risk category (CLL-IPI only); x axis: time in years.

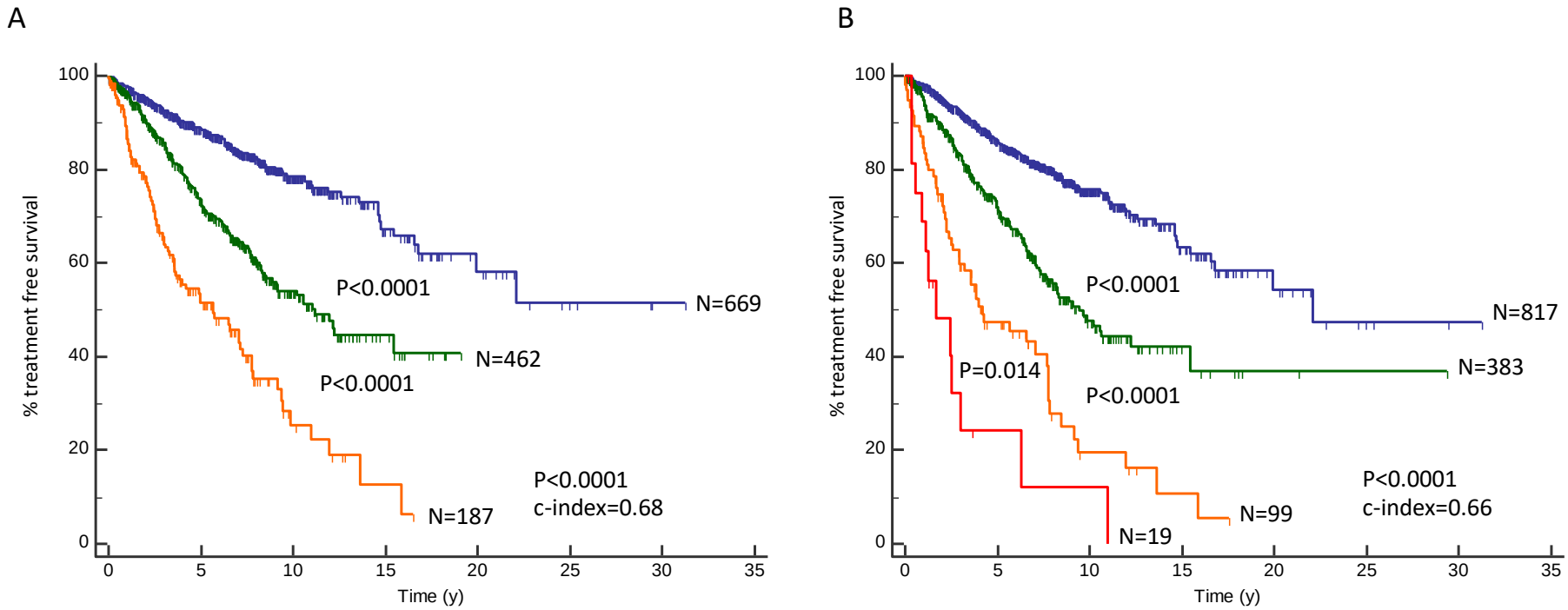
A



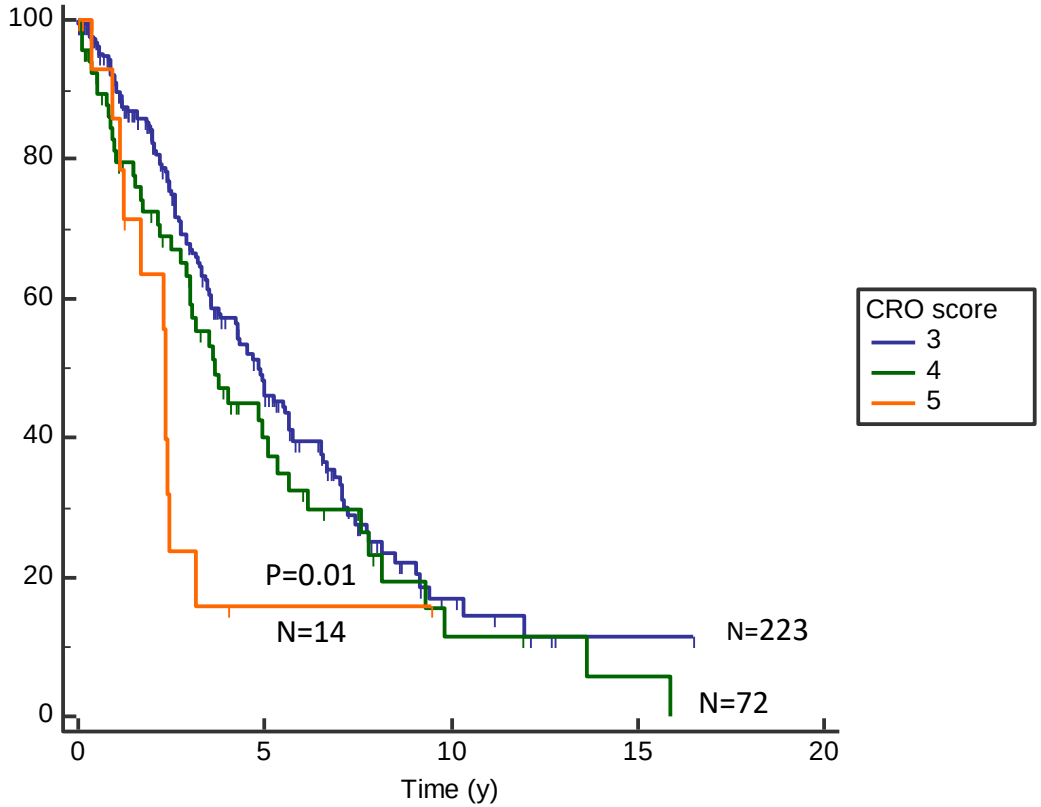
B

	Mayo	Training	O-CLL	Cardiff	Gemelli
Mayo	--	--	--	--	--
Training	0.15	--	--	--	--
O-CLL	0.01	0.25	--	--	--
Cardiff	0.12	0.55	0.66	--	--
Gemelli	0.0007	0.0047	0.15	0.06	--

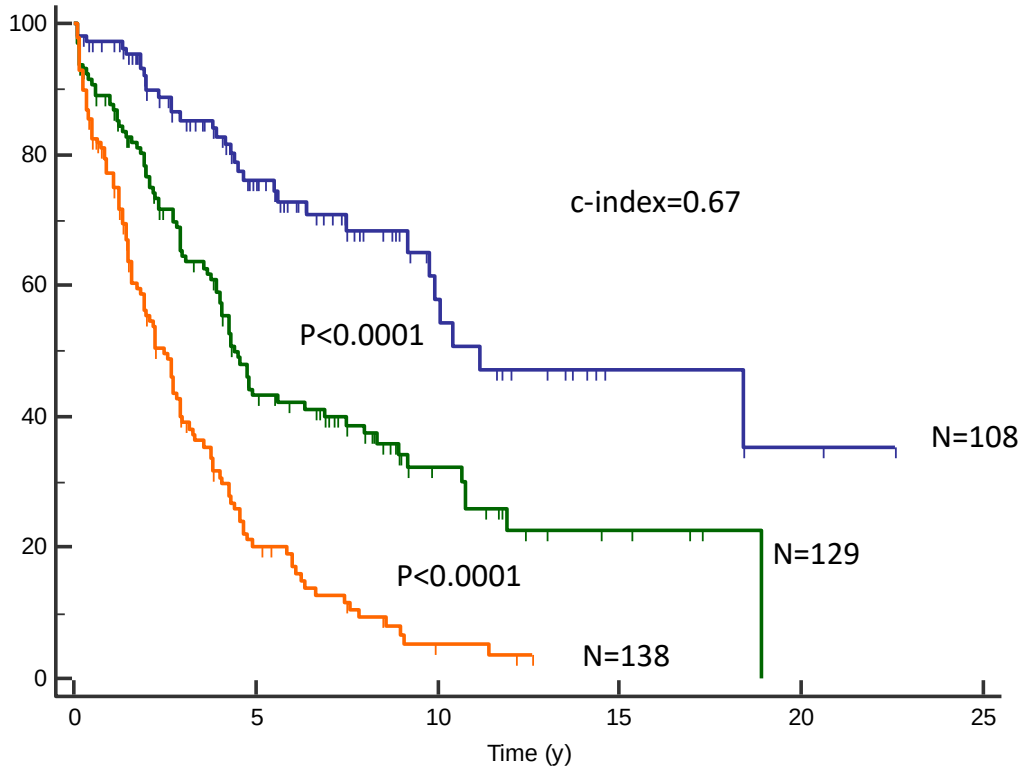
Supplementary Figure 5. Overall treatment free survival in all cohorts. A) Kaplan-Meier curves demonstrating treatment free survival (%, Y-axis) for the training and 4 validation cohorts. B) Statistical significance (P-value) comparing median treatment free survivals between all cohorts.



Supplementary Figure 6. Comparison of CRO score and CLL-IPI in the composite cohort. Kaplan-Meier curves demonstrating treatment free survival (%, Y-axis) in the composite validation cohort determined by the CRO score (A) and CLL-IPI (B). Blue: low-risk category, green: moderate-risk category, orange: high-risk category, red: very high-risk category (CLL-IPI only).



Supplementary Figure 7. Treatment free survival in all high-risk cases determined according to the CRO score. Kaplan-Meier curve demonstrating treatment free survival (%, Y-axis) stratified by CRO score in all high risk cases (N=309) taken from the training and validation cohorts.



Supplementary Figure 8. Application of the CRO score in Rai 1 patients. Kaplan-Meier survival curves demonstrating treatment free survival (%, Y-axis) using the CRO score in Rai I patients applied to a consecutive series of Italian multicenter patients referred to our center for immunocytogenic analyses between 2006 and 2017. Blue: low-risk category, green: moderate-risk category, orange: high-risk category.