

Supplemental Information

CD49d expression is included in a revised 4-factor model predicting outcome in patients with chronic lymphocytic leukemia treated with ibrutinib: a multi-center real-world experience

Running Head: Inclusion of CD49d in a new 4-factor model

- **Supplemental Figures:**
 - **Figure S1. Prognostic impact of the canonical 4-factor;**
 - **Figure S2. Prognostic impact of prior lines of therapy, of β 2M and LDH levels;**
 - **Figure S3. Prognostic impact of modified-4-factor and modified 4-factor-CD49d in patients with 0, or 1 prior lines of therapy;**
 - **Figure S4. Prognostic impact of modified 4-factor-CD49d, and of CD49d expression and TP53 status.**

- **Supplemental Tables:**
 - **Table S1. Clinical features of the retrospective CLL cohort;**
 - **Table S2. Multivariable analyses of PFS and OS (n=401).**

Supplemental Figures

Figure S1. Prognostic impact of the canonical 4-factor. A) Kaplan-Meier curves of canonical 4-factor comparing PFS probabilities of 111 cases with low-risk (low, green line), 160 cases with intermediate-risk (int, orange line), and 130 cases with high-risk (high, red line). B) Kaplan-Meier curves of canonical 4-factor comparing OS probabilities of 111 cases with low-risk (low, green line), 160 cases with intermediate-risk (int, orange line), and 130 cases with high-risk (high, red line). The number of patients in each group is reported; P values refer to log-rank test.

Figure S2. Prognostic impact of prior lines of therapy, of β 2M and LDH levels. A) PFS with ibrutinib by prior lines of therapy of 57 cases treatment naïve (0, green line), 155 cases with 1 prior line (1, dark green line), 107 cases with 2 prior lines (2, light blue line), 42 cases with 3 prior lines (3, purple line), 22 cases with 4 prior lines (4, orange line), and 18 cases with ≥ 5 prior lines (5, red line). B) OS with ibrutinib by prior lines of therapy of 57 cases treatment naïve (0, green line), 155 cases with 1 prior line (1, dark green line), 107 cases with 2 prior lines (2, light blue line), 42 cases with 3 prior lines (3, purple line), 22 cases with 4 prior lines (4, orange line), and 18 cases with ≥ 5 prior lines (5, red line). C) Kaplan-Meier curves of β 2M levels comparing PFS probabilities of 185 cases with β 2M levels < 3.5 mg/L (green line), 98 cases with β 2M levels between 3.5, and 5.0 mg/L (orange line), and 118 cases with β 2M levels ≥ 5.0 mg/L (red line); D) Kaplan-Meier curves of LDH levels comparing PFS probabilities of 208 cases with LDH levels ≤ 250 U/ml (green line), and 193 cases with LDH levels > 250 U/ml (red line). The number of patients in each group is reported; P values refer to log-rank test.

Figure S3. Prognostic impact of modified-4-factor and modified 4-factor-CD49d in patients with 0, or 1 prior lines of therapy. A) Kaplan-Meier curves of modified 4-factor comparing PFS probabilities of 160 cases with low-risk (low, green line), 42 cases with intermediate-risk (int, orange line), and 10 cases with high-risk (high, red line). B) Kaplan-Meier curves of modified 4-factor-CD49d comparing PFS probabilities of 143 cases with low-risk (low, green line), 55 cases with intermediate-risk (int, orange line), and 14 cases with high-risk (high, red line). The number of patients in each group is reported; P values refer to log-rank test.

Figure S4. Prognostic impact of modified 4-factor-CD49d, and of CD49d expression and *TP53* status. A) Kaplan-Meier curves of modified 4-factor-CD49d comparing PFS probabilities of 45 cases with score 0 (0, green line), 137 cases with score 1 (1, dark green line), 141 cases with score 2 (2, light blue line), 65 cases with score 3 (3, purple line), and 13 cases with score 4 (4, orange line). B) Kaplan-Meier curves of CD49d expression and *TP53* status comparing PFS probabilities of 110 cases with CD49d-low expression and *TP53* wild-type status (no deletion and mutations) (CD49d-low_TP53wt, green line), 23 cases with CD49d-low expression and *TP53* disrupted (deletion and/or mutations) (CD49d-low_TP53dis, dark green line), 199 cases with CD49d-high expression and *TP53* wild-type status (CD49d-high_TP53wt, light blue line), and 69 cases with CD49d-high expression and *TP53* disrupted (CD49d-high_TP53dis, purple line). The number of patients in each group is reported; P values refer to log-rank test.

Table S1. Clinical features of the retrospective CLL cohort

Parameter	Category	Total cohort n=401	
		N	%
Age (y)	<65	119	29.7
	≥65	282	70.3
Gender	Female	139	34.7
	Male	262	65.3
Prior Lines of therapy ^a	0	57	14.2
	1	155	40.5
	2	107	27.9
	3	42	11.0
	4	22	5.7
	5	18	4.7
β2 microglobulin (mg/L)	<5	283	70.6
	≥5	118	29.4
Lactate dehydrogenase (U/ml)	≤250	208	51.9
	>250	193	48.1
TP53	wild-type (wt)	211	52.6
	Deleted only	19	4.7
	Mutated only	80	20.0
	Mutated and deleted	91	22.7
CD49d	Low	133	33.2
	High/bimodal	268	66.8
IGHV	Mutated (M)	99	24.7
	Unmutated (UM)	292	72.8
	Missing	10	2.5

IGHV mutated, <98% identity with germ line; IGHV unmutated, ≥98% identity with germ line.

^a number of prior lines of therapy before ibrutinib treatment.

Table S2. Multivariable analyses of PFS and OS (n=401)

PFS

	UVA				MVA			
	HR	LCI	UCI	P	HR	LCI	UCI	P
CD49d (high+bimodal)	1.75	1.24	2.48	0.0016	1.51	1.06	2.15	0.0232
Modified 4-factor intermediate^a	1.78	1.23	2.58	0.0023	1.74	1.20	2.53	0.0033
Modified 4-factor high^a	3.21	2.23	4.63	<0.0001	2.96	2.05	4.29	<0.0001

Notes and abbreviations: CD49d high+bimodal according to 30% cutoff; bimodal cases were included in the high category as reported in ref. n. 4; PFS, progression free survival from ibrutinib start; UVA, univariable analysis; MVA, multivariable analysis; HR, Hazard Ratio; CI, confidence interval; LCI, 95% lower CI; UCI, 95% Upper CI; -: not used in

^aTreated as categorical variables respect 4-factor-modified low group.

OS

	UVA				MVA			
	HR	LCI	UCI	P	HR	LCI	UCI	P
CD49d (high+bimodal)	2.30	1.44	3.68	0.0005	2.0257	1.2566	3.2656	0.0038
Modified 4-factor intermediate^a	1.61	1.03	2.54	0.0387	1.5762	1.0004	2.4832	0.0498
Modified 4-factor high^a	2.47	1.59	3.85	0.0001	2.1096	1.3418	3.3166	0.0012

Notes and abbreviations: CD49d high+bimodal according to 30% cutoff; bimodal cases were included in the high category as reported in ref. n. 4; PFS, progression free survival from ibrutinib start; UVA, univariable analysis; MVA, multivariable analysis; HR, Hazard Ratio; CI, confidence interval; LCI, 95% lower CI; UCI, 95% Upper CI; -: not used in

^aTreated as categorical variables respect 4-factor-modified low group.

Multivariable analyses of OS (modified 4-factor variables and CD49d; n=401)

	UVA				MVA				Bootstrapping selection (%)
	HR	LCI	UCI	P	HR	LCI	UCI	P	
Prior Lines of therapy (≥ 1)	1.69	1.15	2.46	0.0068	1.70	1.16	2.50	0.0065	88.3
$\beta 2M$ (high)	1.67	1.13	2.45	0.0094	1.47	1.00	2.17	0.0510	62.9
LDH (high)	1.77	1.22	2.59	0.0030	ni				43.2
TP53 disruption (del17p and TP53 mut)	1.52	1.02	2.25	0.0382	1.53	1.03	2.28	0.0366	75.8
CD49d (low vs high+bimodal)	2.30	1.44	3.68	0.0005	2.14	1.34	3.42	0.0015	73.1

Notes and abbreviations: b2 microglobulin high, > 5 mg/L; LDH high, >250 U/ml; CD49d high+bimodal according to 30% cutoff; bimodal cases were included in the high category as reported in ref. n. 4; PFS, progression free survival from ibrutinib start; MVA, multivariable analysis; HR, Hazard Ratio; CI, confidence interval; LCI, 95% lower CI; UCI, 95% Upper CI; ni: not included in the final model.

Figure S1

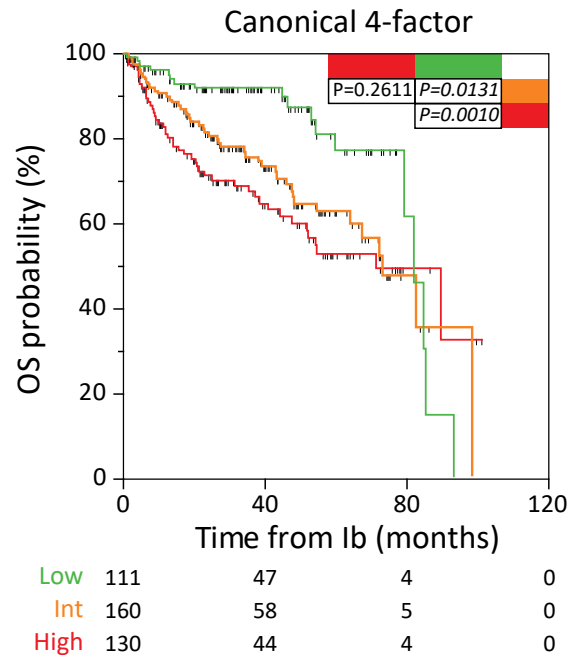
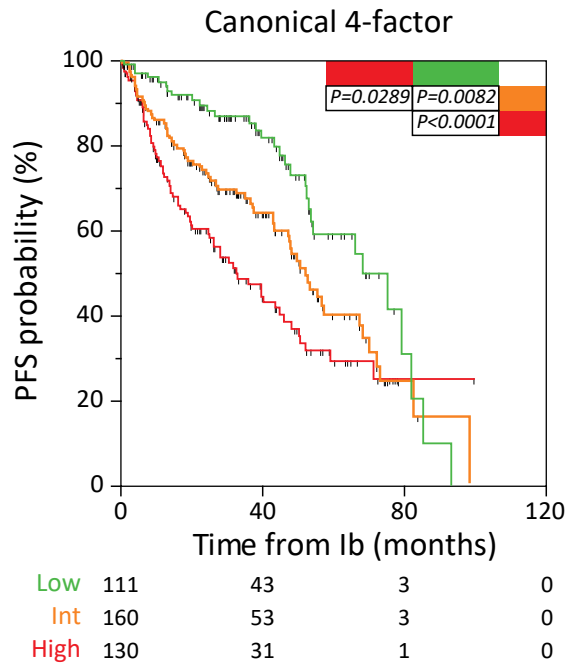


Figure S2

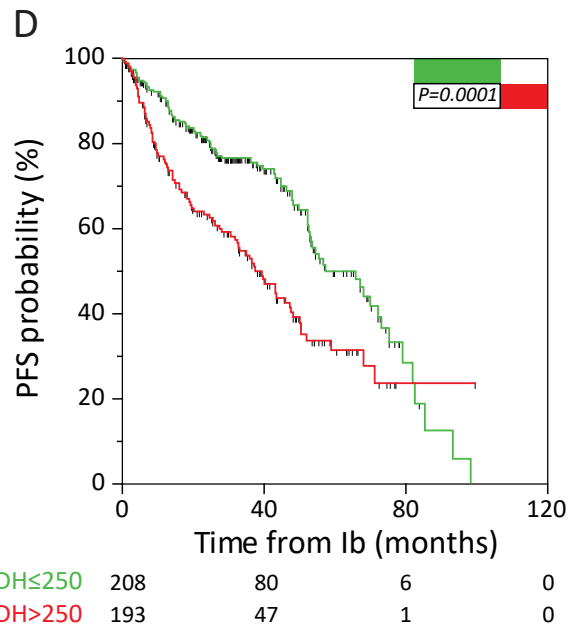
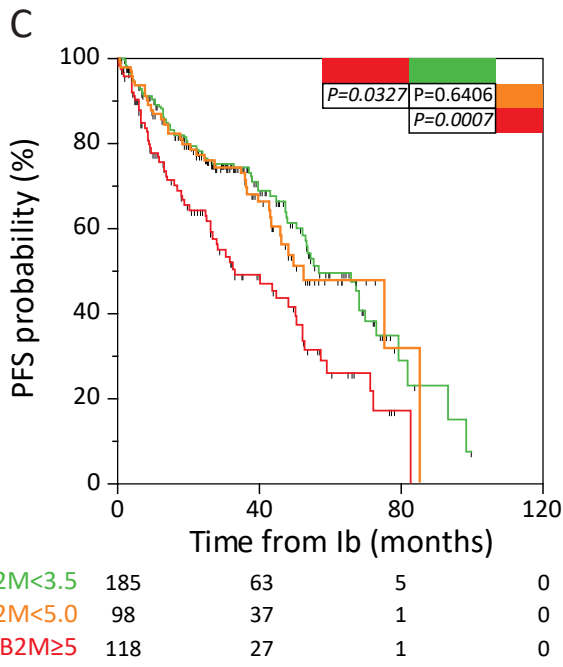
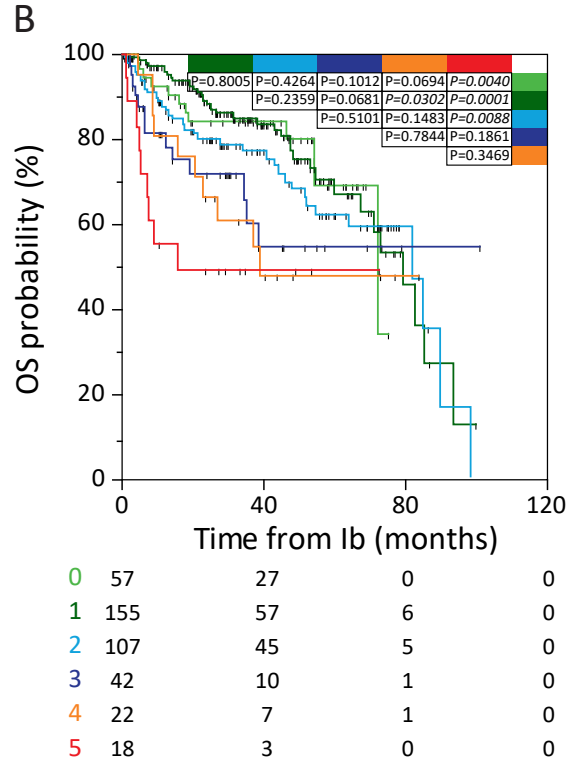
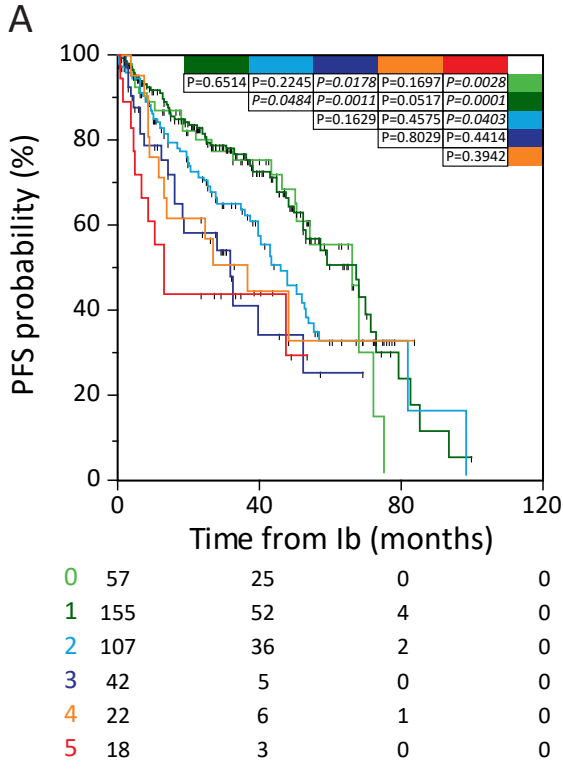


Figure S3

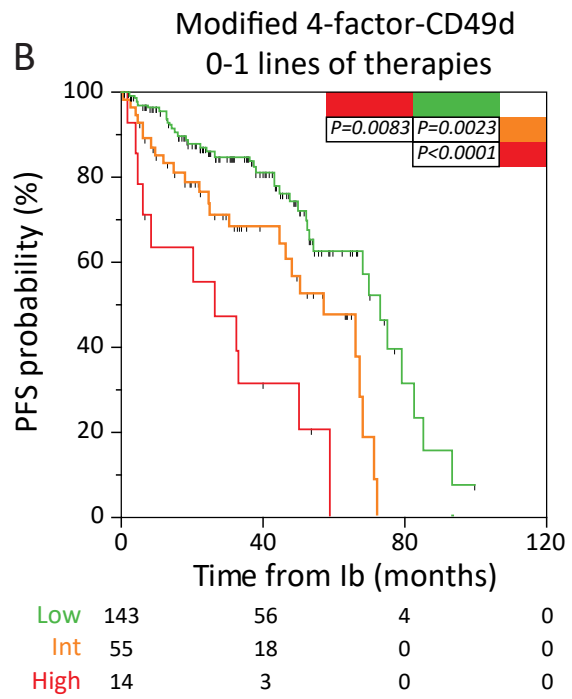
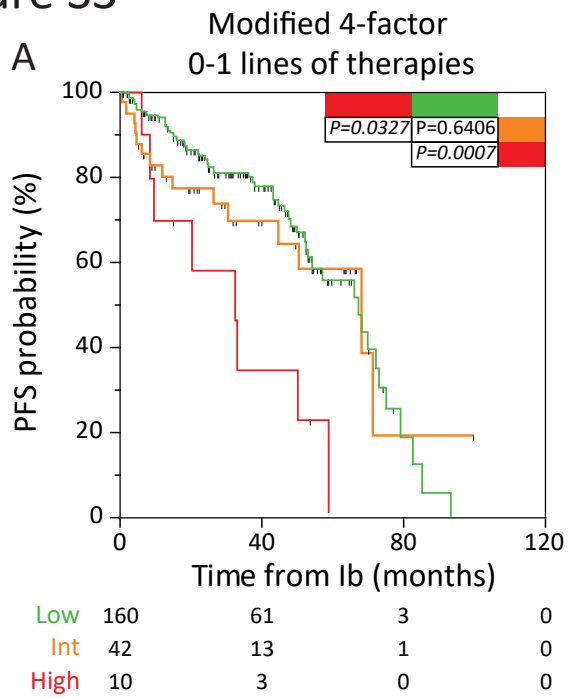


Figure S4

