

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

Riccardo Bomben<sup>1</sup> | Antonella Zucchetto<sup>1</sup> | Roberta Laureana<sup>2</sup> | Annalisa Chiarenza<sup>3</sup> | Jacopo Olivieri<sup>4</sup> | Erika Tissino<sup>1</sup> | Francesca M. Rossi<sup>1</sup> | Filippo Vit<sup>1</sup> | Tamara Bittolo<sup>1</sup> | Robel Papotti<sup>1</sup> | Federico Pozzo<sup>1</sup> | Annalisa Gaglio<sup>1</sup> | Massimo Degan<sup>1</sup> | Jerry Polesel<sup>5</sup> | Roberto Marasca<sup>6,7</sup> | Andrea Visentin<sup>8</sup> | Riccardo Moia<sup>9</sup> | Idanna Innocenti<sup>10</sup> | Candida Vitale<sup>11</sup> | Roberta Murru<sup>12</sup> | Marzia Varettoni<sup>13</sup> | Agostino Tafuri<sup>14</sup> | Francesco Zaja<sup>15</sup> | Massimiliano Postorino<sup>2</sup> | Enrica A. Martino<sup>16</sup> | Adalgisa Condoluci<sup>17</sup> | Davide Rossi<sup>17</sup> | Antonio Cuneo<sup>18</sup> | Francesco Di Raimondo<sup>3</sup> | Paolo Sportoletti<sup>19</sup> | Ilaria Del Giudice<sup>20</sup> | Robin Foà<sup>20</sup> | Francesca R. Mauro<sup>20</sup> | Marta Coscia<sup>11</sup> | Luca Laurenti<sup>10</sup> | Gianluca Gaidano<sup>9</sup> | Livio Trentin<sup>8</sup> | Maria I. Del Principe<sup>2</sup> | Massimo Gentile<sup>16,21</sup> | Valter Gattei<sup>1</sup>

Correspondence: Riccardo Bomben ([rbomben@cro.it](mailto:rbomben@cro.it)); Valter Gattei ([vgattei@cro.it](mailto:vgattei@cro.it))

Chronic lymphocytic leukemia (CLL) is a malignancy of mature clonal B lymphocytes that accumulate in blood, bone marrow, and lymphoid tissues.<sup>1</sup> One of the most important key players in the pathobiology and progression of CLL is the B-cell receptor (BCR) whose activation

supports growth and survival of CLL cells.<sup>2</sup> For this reason, the use of Bruton's tyrosine kinase inhibitors (BTKi) including ibrutinib emerged as one of the most effective treatment options for both naïve (TN) and relapsed/refractory (RR) CLL.<sup>2</sup> Treatment with BTKi often results

<sup>1</sup>Clinical and Experimental Onco-Haematology Unit, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy

<sup>2</sup>Division of Haematology, University of Tor Vergata, Rome, Italy

<sup>3</sup>Division of Hematology, Policlinico, Department of Surgery and Medical Specialties, University of Catania, Catania, Italy

<sup>4</sup>Clinica Ematologica, Centro Trapianti e Terapie Cellulari "Carlo Melzi" DISM, Azienda Ospedaliera Universitaria S. Maria Misericordia, Udine, Italy

<sup>5</sup>Unit of Cancer Epidemiology, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano, Italy

<sup>6</sup>Hematology Unit, Department of Oncology and Hematology, Azienda-Ospedaliero Universitaria (AOU) of Modena, Policlinico, Modena, Italy

<sup>7</sup>Department of Medical and Surgical Sciences, University of Modena e Reggio Emilia, Emilia-Romagna, Italy

<sup>8</sup>Hematology unit, Department of Medicine, University of Padova, Padova, Italy

<sup>9</sup>Division of Hematology, Department of Translational Medicine, Università del Piemonte Orientale, Novara, Italy

<sup>10</sup>Fondazione Universitaria Policlinico A Gemelli di Roma, Roma, Italy

<sup>11</sup>A.O.U. Città della Salute e della Scienza di Torino, Torino, Italy

<sup>12</sup>Hematology and Stem Cell Transplantation Unit, Ospedale A. Businco, ARNAS G. Brotzu, Cagliari, Italy

<sup>13</sup>Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy

<sup>14</sup>Department of Clinical and Molecular Medicine and Hematology, Sant'Andrea - University Hospital - Sapienza, University of Rome, Rome, Italy

<sup>15</sup>Department of Medical, Surgical and Health Sciences, University of Trieste, Trieste, Italy

<sup>16</sup>Hematology Unit, Azienda Ospedaliera Annunziata, Cosenza, Italy

<sup>17</sup>Oncology Institute of Southern Switzerland and Institute of Oncology Research, Bellinzona, Switzerland

<sup>18</sup>Hematology Section, Department of Medical Sciences, University of Ferrara, Ferrara, Italy

<sup>19</sup>Centro di Ricerca Emato-Oncologica (CREO), Dipartimento di Medicina e Chirurgia, Università di Perugia, Perugia, Italy

<sup>20</sup>Hematology, Department of Translational and Precision Medicine, 'Sapienza' University, Rome, Italy

<sup>21</sup>Department of Pharmacy, Health and Nutritional Science, University of Calabria, Rende, Italy

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in an increase blood lymphocytosis driven by the release of cells from lymph nodes due to impaired interaction with the microenvironment.<sup>2-4</sup> One of the key molecules of these interactions is the integrin VLA-4 which mediates both cell-cell and cell-matrix interactions playing a crucial role in the retention of CLL cells in tissue-sites thus protecting them from proapoptotic signal.<sup>3,5-7</sup> In keeping with these observations, high expression of the VLA-4 integrin alpha chain CD49d ( $\geq 30\%$  positive cells), or expression of CD49d according to a bimodal pattern (i.e., concurrent CD49d-positive and CD49d-negative subpopulations, irrespective of the 30% cutoff) identifies CLL cases with reduced recirculation lymphocytosis, inferior nodal response, and shorter progression-free survival (PFS) in the ibrutinib setting.<sup>4,5</sup>

In addition to CD49d evaluation, a 4-factor model has been proposed to identify patients at high risk of treatment failure and death during ibrutinib therapy, afterward validated in real-world, which included the TN/RR status, the levels of  $\beta 2$ -microglobulin ( $\beta 2M$ ) and lactate dehydrogenase (LDH) serum concentration, and the TP53 disruption status.<sup>8,9</sup> In this context, as well as in other clinical studies on CLL, the TP53 disruption category includes CLL cases with either concurrent TP53 mutation and deletion, or only one of the lesions.<sup>1,8,9</sup> Recently, the prognostic impact of TP53 disruption in ibrutinib-treated CLL has been refined by demonstrating that only cases with the concomitant presence of TP53 deletion and mutations, did not gain maximum benefit from this therapy.<sup>10,11</sup>

The aim of this study is to integrate these observations in a comprehensive scoring system for a better management of ibrutinib-treated patients.

The study is a retrospective/multicenter analysis of 401 CLL patients treated with ibrutinib in the current clinical practice (12/2013-03/2022; approvals IRB-05-2010/IRB-05-2015; CRO Aviano). All CLL cases were characterized for CD49d expression, as reported previously.<sup>4-7,12</sup> TP53 disruption was simultaneously evaluated by FISH (17p deletion, del17p) and next-generation sequencing (TP53 mutations), as reported.<sup>10,12</sup> PFS was calculated from the date of ibrutinib initiation to progression and/or death (death for OS) or last follow-up. Among 401 patients (282 aged  $\geq 65$  years at ibrutinib start), 112 died and 169 progressed after median follow-up of 29.9 months (95% confidence interval [CI]: 26.7-34.1 months) and 26.5 months (95% CI: 23.8-29.6 months) from ibrutinib start, respectively. See Supporting Information S1: Table S1 for further details. The canonical 4-factor prediction model was computed as reported:<sup>8</sup> TP53 aberration (del17p and/or TP53 mutations) 1 point,  $>0$  prior treatment, 1 point, LDH  $> 250$  U/L, 1 point,  $\beta 2M \geq 5$  mg/L, 1 point). Patients were stratified into low-risk (score 0-1), intermediate-risk (score 2), high-risk (score 3-4) groups.<sup>8</sup>

Applying this model we were able to identify 111 low-risk, 160 intermediate-risk, and 130 high-risk cases.<sup>8,9</sup> Accordingly, low-risk patients presented a significant longer PFS (median 75.0 months) than patients in the intermediate-risk (median 52.1 months;  $p = 0.0082$ ) and high-risk groups (median 32.9 months;  $p < 0.0001$ , Supporting Information S1: Figure S1A). Low-risk patients also presented a significant longer OS (median 81.7 months) than patients in the intermediate-risk and high-risk groups ( $p = 0.0131$ , and  $p = 0.0010$ , respectively), while no significant differences were found between intermediate-risk (median 72.8 months), and high-risk patients (median 71.0 months,  $p = 0.2611$ , Supporting Information S1: Figure S1B).

Based on previous findings on the clinical impact of TP53 disruption in the ibrutinib setting,<sup>10,11</sup> we developed a modified 4-factor model by considering only patients with a concomitant TP53 deletion and mutation as TP53 disrupted ( $n = 91$ ; Supporting Information S1: Table S1). Moreover, in keeping with a previous report,<sup>13</sup> as no difference in PFS and/or OS was found between untreated ( $n = 57$ ) and 1-line prior treated patients ( $n = 155$ ;  $p = 0.6514$  or  $p = 0.8005$ , respectively; Supporting Information S1: Figure S2AB), these two groups were

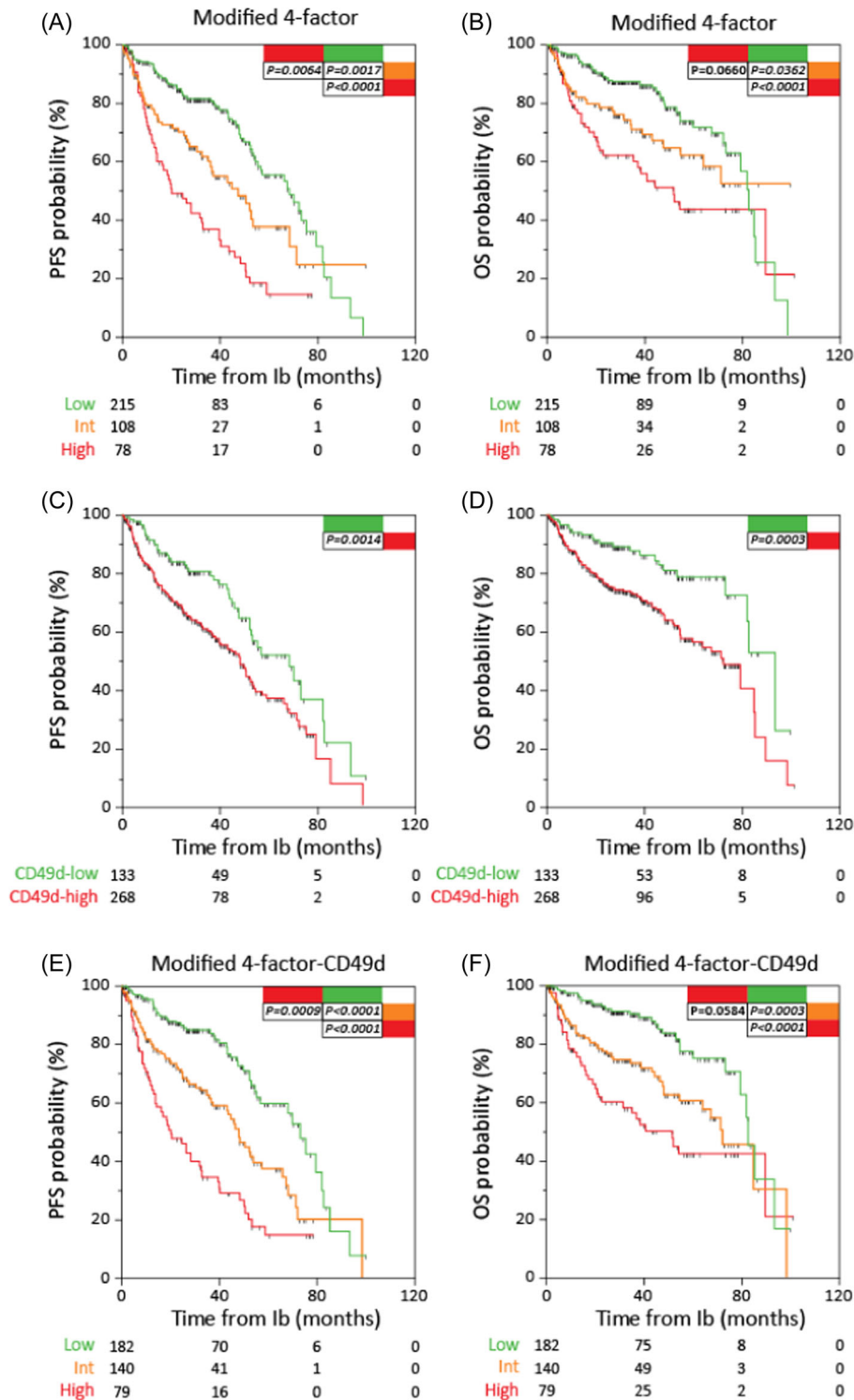
combined ( $n = 212$ ) and separated from patients with  $>1$  line prior therapy ( $n = 189$ ; Supporting Information S1: Table S1). Therefore, one point was assigned to patients with concomitant TP53 deletion and mutation, and patients treated with  $>1$  line of prior therapy.<sup>8</sup> Conversely, the cutoffs for  $\beta 2M$  and LDH, also confirmed in our series (Supporting Information S1: Figure S1C and S1D), were maintained as in the original 4-factor study.<sup>8</sup>

Accordingly, in this modified 4-factor model, 215 low-risk patients presented significantly longer PFS (median 67.9 months) than 108 intermediate-risk (median 47.2 months;  $p = 0.0017$ ), and 78 high-risk patients (median 20.2 months;  $p < 0.0001$ ; Figure 1A). The latter model outperformed the canonical 4-factor (C-indices 0.646, 95% CI: 0.604-0.688, vs. 0.628, 95% CI: 0.586-0.670;  $p < 0.0001$ ). The data were even more evident in the OS setting where C-index for the canonical 4-factor (0.616) was significantly inferior than C-index for the modified 4-factor (0.639;  $p < 0.0001$ ; Figures S1B and 1B); low-risk patients had significant longer OS (median 82.5 months) than intermediate-risk (median 81.2 months;  $p = 0.0362$ ), and high-risk (median 51.5 months;  $p < 0.0001$ ) patients (Figure 1B). Circumscribing the analysis to patients with 0-1 lines of prior therapy, again high-risk patients experienced a shorter PFS than low-risk and intermediate-risk patients ( $p = 0.0007$ , and  $p = 0.0327$ , respectively; Supporting Information S1: Figure S3A).

In our cohort, CD49d-high CLL cases (expression  $\geq 30\%$  and/or bimodal,  $n = 268$ ) had shorter PFS and OS intervals ( $p = 0.0014$  and  $p = 0.0003$ , respectively) than CD49d-low cases ( $n = 133$ ; Figure 1CD). As shown in Supporting Information S1: Table S2, CD49d remained an independent prognostic factor for PFS ( $p = 0.0232$ ) and OS ( $p = 0.0038$ ) in multivariable models adjusted with the inclusion of the modified 4-factor intermediate-risk ( $p = 0.0033$ , PFS;  $p = 0.0498$ , OS) and high-risk ( $p < 0.0001$ , PFS;  $p = 0.0012$ , OS) groups. We then re-analyzed the individual parameters of the modified 4-factor score together with CD49d expression. According to novel multivariable analyses, LDH was excluded as an independent predictor in favor of CD49d expression (PFS,  $p = 0.0068$ ; OS,  $p = 0.0015$ ; Table 1 and Supporting Information S1: Table S2). Consistently, bootstrapping analyses, selected CD49d (PFS, 72.2%; OS, 73.1%) more frequently compared to LDH (PFS, 39.4%; OS, 43.2%) (Table 1 and Supporting Information S1: Table S2).

According to these results, a novel 4-factor model excluding LDH data in favor of CD49d expression data (4-factor-CD49d) identified 45 scored 0, 137 scored 1, 141 scored 2, 65 scored 3, and 13 scored 4 patients. Based on PFS data, patients with score 0 and 1 presented similar PFS intervals with no significant differences ( $p = 0.7328$ ); similar behavior was observed for patients with scores 3 and 4 ( $p = 0.7877$ ) who presented the worst outcome, while cases with score 2, showing intermediate outcomes, differed significantly from all other scores (Supporting Information S1: Figure S4A). Patients were then stratified into three groups with significantly different risk for PFS: score 0-1, low-risk ( $n = 182$ ); score 2, intermediate-risk ( $n = 140$ ); score 3-4, high-risk ( $n = 79$ ; Figure 1E). The inclusion of CD49d into this novel 4-factor model improved its PFS prediction capability (C-index = 0.668, 95% CI: 0.627-0.709;  $p < 0.0001$  vs. both the canonical 4-factor and the 4-factor-modified). Similar results were obtained when considering OS as clinical readout (Figure 1F; C-index = 0.670, 95% CI: 0.621-0.719;  $p < 0.0001$  vs. both the canonical 4-factor and the 4-factor-modified). Focusing only on patients with 0-1 prior lines of therapies, high-risk patients ( $n = 14$ ) presented significantly shorter PFS than intermediate-risk ( $n = 55$ ) and low-risk ( $n = 143$ ) cases ( $p = 0.0083$ , and  $p < 0.0001$ ; respectively; Supporting Information S1: Figure S3B).

In this scenario, we can speculate that CLL cells with the concomitant presence of the integrin VLA-4, which can promote the retention of CLL cells at tissue sites through binding to its specific ligands,<sup>3,4</sup> and TP53



**FIGURE 1** Prognostic impact of the modified 4-factor and CD49d expression. (A) Kaplan-Meier curves of modified 4-factor comparing progression-free survival (PFS) probabilities of 215 cases with low-risk (low, green line), 108 cases with intermediate-risk (int, orange line), and 78 cases with high-risk (high, red line); (B) Kaplan-Meier curves of modified 4-factor comparing overall survival (OS) probabilities of 215 cases with low-risk (low, green line), 108 cases with intermediate-risk (int, orange line), and 78 cases with high-risk (high, red line). (C) Kaplan-Meier curves of CD49d expression (low vs. high) comparing PFS probabilities of 133 cases with CD49d low expression (CD49d-low, green line), and 268 cases with CD49d high or bimodal expression (CD49d-high, red line); (D) Kaplan-Meier curves of CD49d expression (low versus high) comparing OS probabilities of 133 cases with CD49d low expression (CD49d-low, green line), and 268 cases with CD49d high or bimodal expression (CD49d-high, red line). (E) Kaplan-Meier curves of modified 4-factor-CD49d comparing PFS probabilities of 182 cases with low-risk (low, green line), 140 cases with intermediate-risk (int, orange line), and 79 cases with high-risk (high, red line); (F) Kaplan-Meier curves of modified 4-factor-CD49d comparing OS probabilities of 182 cases with low-risk (low, green line), 140 cases with intermediate-risk (int, orange line), and 79 cases with high-risk (high, red line). The number of patients in each group is reported;  $p$  values refer to log-rank test.

**TABLE 1** Multivariable analyses of PFS (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	1.25	2.29	0.0007	1.73	1.27	2.36	0.0005	88.1
β2M (high)	1.83	1.33	2.51	0.0002	1.61	1.17	2.22	0.0035	63.7
LDH (high)	1.86	1.37	2.53	0.0001	ni				39.4
TP53 disruption (del17p and TP53 mut)	1.72	1.25	2.37	0.0009	1.72	1.24	2.37	0.0011	76.9
CD49d (high+bimodal)	1.75	1.24	2.48	0.0016	1.62	1.14	2.31	0.0068	72.2

Note: β2 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. Bold values refer to significant p-values.

Abbreviations: CI, confidence interval; HR, hazard ratio; ni, not included in the final model; PFS, progression-free survival from ibrutinib start; LCI, 95% lower CI; MVA, multivariable analysis; UCI, 95% upper CI; UVA, univariable analysis.

disruption, itself responsible for genetic instability,<sup>14</sup> may be particularly prone to survive/proliferate and accumulate genetic lesions possibly responsible for resistance to ibrutinib. Consistently, the concomitant presence of TP53 disruption and high CD49d expression selected a subgroup of patient with a particularly high risk of progression, even when compared with cases with TP53 disruption or CD49d expression alone (Supporting Information S1: Figure S4B).

Although CD49d is not routinely tested and is not recommended by the iwCLL guidelines,<sup>1</sup> it turned out to be a biomarker that can improve prognostic stratification of patients for both PFS and OS.<sup>4,5</sup> In this regard, the C-indices of models that included CD49d approached values of 0.7, a threshold considered necessary to confer utility at the individual patient level.<sup>15</sup>

This scoring model, generated from patients treated with ibrutinib, should be confirmed in the context of second generation BTKi. Based on the stratification proposed here, patients in the high-risk group could be considered for combination therapies and/or enrollment in clinical trials. Further validation in independent cohorts is needed.

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## AUTHOR CONTRIBUTIONS

Riccardo Bomben designed the study, interpreted data, and wrote the manuscript. Antonella Zucchetto, Erika Tissino, Francesca M. Rossi, Filippo Vit, Tamara Bittolo, Robel Papotti, Federico Pozzo, Annalisa Gaglio, Massimo Degan performed and interpreted molecular studies, and contributed to data interpretation. Jerry Polesel performed statistical analyses. Roberta Laureana, Annalisa Chiarenza, Jacopo Olivieri, Roberto Marasca, Andrea Visentin, Riccardo Moia, Idanna Innocenti, Candida Vitale, Roberta Murru, Marzia Varettoni, Agostino Tafuri, Francesco Zaja, Massimiliano Postorino, Enrica A. Martino, Adalgisa Condoluci, Davide Rossi, Antonio Cuneo, Francesco Di Raimondo, Paolo Sportoletti, Ilaria Del Giudice, Robin Foà, Francesca R. Mauro, Marta Coscia, Luca Laurenti, Gianluca Gaidano, Livio Trentin, Maria I. Del Principe, Massimo Gentile collected clinical data and contributed to data interpretation. Valter Gattei designed the study, interpreted data, and wrote the manuscript.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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## SUPPORTING INFORMATION

Additional supporting information can be found in the online version of this article.

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