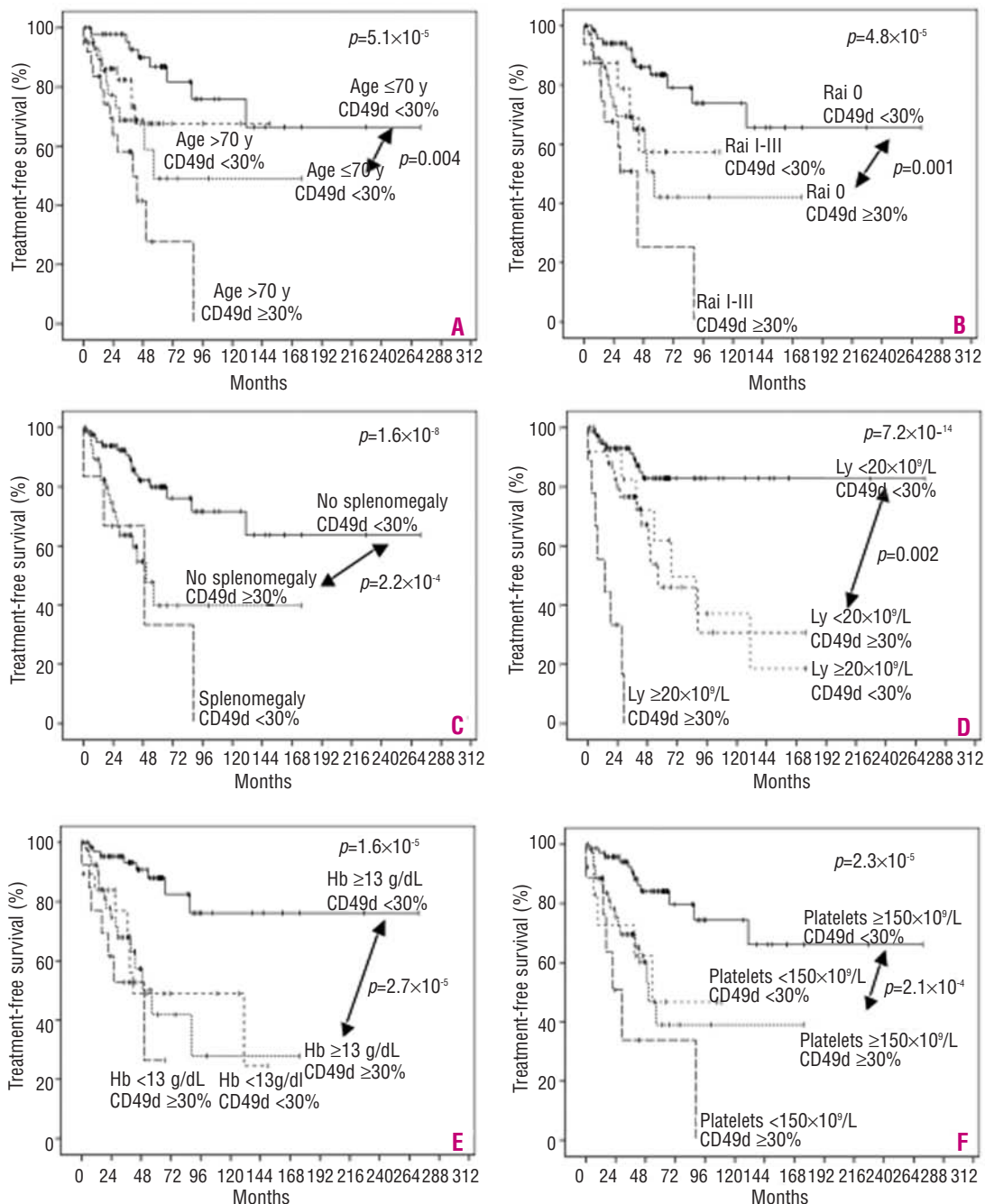


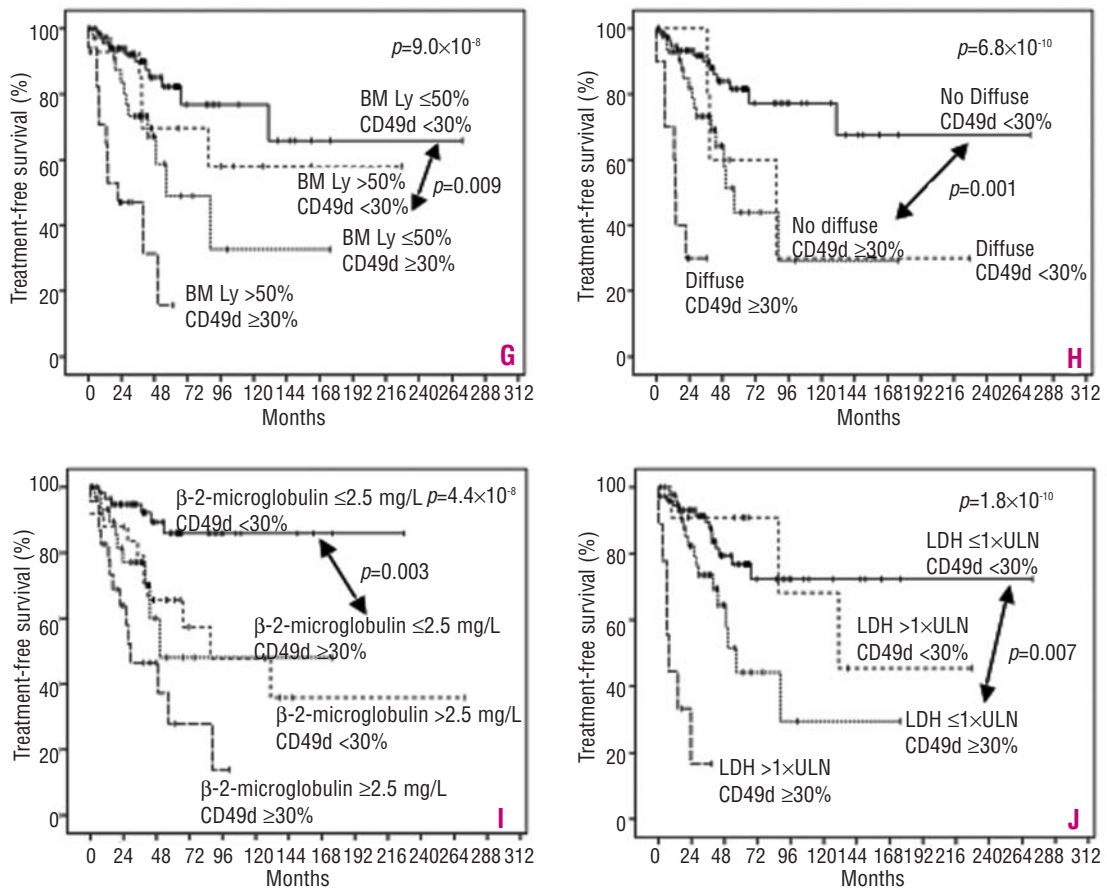
CD49d expression is an independent risk factor of progressive disease in early stage chronic lymphocytic leukemia

Davide Rossi,¹ Antonella Zucchetto,² Francesca Maria Rossi,² Daniela Capello,¹ Michaela Cerri,¹ Clara Deambrogi,¹ Stefania Cresta,¹ Silvia Rasi,¹ Lorenzo De Paoli,¹ Chiara Lobetti Bodoni,³ Pietro Bulian,² Giovanni Del Poeta,⁴ Marco Ladetto,³ Valter Gattei,² and Gianluca Gaidano¹

¹Division of Hematology, Department of Clinical and Experimental Medicine and BRMA, Amedeo Avogadro University of Eastern Piedmont and Azienda Ospedaliero-Universitaria Maggiore della Carità, Novara; ²Clinical and Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico, I.R.C.C.S., Aviano; ³Division of Hematology, Department of Experimental Medicine and Oncology, University of Turin, Turin and ⁴Department of Hematology, S. Eugenio Hospital and University of Tor Vergata, Rome, Italy

Citation: Rossi D, Zucchetto A, Rossi FM, Capello D, Cerri M, Deambrogi C, Cresta S, Rasi S, De Paoli L, Lobetti Bodoni C, Bulian P, Del Poeta G, Ladetto M, Gattei V, and Gaidano G. CD49d expression is an independent risk factor of progressive disease in early stage chronic lymphocytic leukemia. *Haematologica* 2008. doi: 10.3324/haematol.13103





Supplementary Figure S1. Survival curves for treatment free survival (TFS) according to CD49d expression in combination with clinical risk factors. CD49d expression $\geq 30\%$ segregated a group of CLL displaying short TFS despite being characterized by age < 70 year (A), Rai 0 stage (B), absence of splenomegaly (C), lymphocyte count $< 20 \times 10^9/L$ (D), Hb ≥ 13 g/dl (E), platelets $\geq 150 \times 10^9/L$ (F), bone marrow lymphocytes $< 50\%$ (G), non-diffuse bone marrow pattern (H), β -2-microglobulin ≤ 2.5 mg/L (I), LDH $\leq 1 \times$ ULN (J). Patients displaying the worst TFS were identified by the combination of CD49d expression $> 30\%$ and one of the following: lymphocyte count $\geq 20 \times 10^9/L$ ($p=0.001$) (D), bone marrow lymphocytes $> 50\%$ ($p=0.010$) (G), β -2-microglobulin > 2.5 mg/L ($p=0.030$) (I), or LDH $> 1 \times$ ULN ($p=0.001$) (J).

Supplementary Table S1. Biological and clinical characteristics of the Binet A CLL cohort at diagnosis.

Characteristics at CLL diagnosis

| | |
|--|------------------|
| Biological variables | |
| IGHV homology >98% | 42/137 (30.7%) |
| Normal FISH | 37/140 (26.4%) |
| Number of FISH lesions >1 | 22/140 (15.7%) |
| del13q14 | 79/140 (56.4%) |
| del13q14 only | 59/140 (42.1%) |
| +12 | 27/140 (19.3%) |
| del11q22-q23 | 7/140 (5.0%) |
| del17p13 | 11/140 (7.9%) |
| del17p13/del11q22-q23/+12 | 43/140 (30.7%) |
| TP53 mutated | 12/140 (8.6%) |
| TP53 deleted and/or mutated | 15/140 (10.7%) |
| CD38 \geq 30% | 32/140 (22.9%) |
| ZAP70 \geq 20% | 38/140 (28.4%) |
| CD49d \geq 30% | 54/140 (38.6%) |
| Clinical variables | |
| Age (years) | 69 (62-76) |
| Male:female | 73:67 |
| ECOG PS >1 | 15/140 (10.7%) |
| Rai stage | |
| 0 | 108/140 (77.1%) |
| I | 24/140 (17.1%) |
| II | 5/140 (3.6%) |
| III | 3/140 (2.1%) |
| Lymph node | 26/140 (18.6%) |
| Largest lymph node \geq 3 cm | 2/140 (1.4%) |
| Splenomegaly | 8/140 (5.7%) |
| Peripheral blood lymphocytes ($\times 10^9/L$) | 9.5 (6.0-16.8) |
| Hb (g/dL) | 13.8 (13.1-14.8) |
| Platelets ($\times 10^9/L$) | 206 (168-241) |
| Bone marrow lymphocytes (%) | 35 (20-50) |
| Diffuse bone marrow pattern | 16/140 (11.6%) |
| β -2-microglobulin (mg/L) | 2.2 (1.7-2.9) |
| LDH (U/L) | 349 (289-412) |
| Alkaline phosphatase (U/L) | 157 (127-177) |
| Albumin (g/L) | 43 (40-46) |
| Direct antiglobulin test | 11/87 (12.6%) |
| Serum monoclonal component | 23/140 (16.4%) |
| IgG (g/L) | 9.7 (8.1-11.6) |
| IgA (g/L) | 1.3 (0.9-2.0) |
| IgM (g/L) | 0.5 (0.3-0.8) |
| CD4 ⁺ lymphocytes ($\times 10^9/L$) | 0.923 (0.5-1.6) |

^a25th-75th percentiles are reported in parentheses for continuous variables.

Supplementary Table S2. Distribution of biological and clinical characteristics at diagnosis of Binet A CLL according to CD49d expression.^a

| Characteristics at CLL diagnosis | CD49d ≥30% | CD49d <30% | p |
|--|------------------|------------------|----------------------|
| Biological variables | | | |
| IGHV homology >98% | 22/54 (40.7%) | 20/83 (24.1%) | 0.061 |
| Normal FISH | 14/54 (25.9%) | 23/86 (26.7%) | 1.000 |
| Number of FISH lesions >1 | 10/54 (18.5%) | 12/86 (14.0%) | 0.628 |
| del13q14 | 24/54 (44.4%) | 55/86 (64.0%) | 0.037 |
| del13q14 only | 16/54 (29.6%) | 43/86 (50.0%) | 0.028 |
| +12 | 17/54 (31.5%) | 10/86 (11.6%) | 0.007 |
| del11q22-q23 | 2/54 (3.7%) | 5/86 (5.8%) | 0.707 |
| del17p13 | 7/54 (13.0%) | 4/86 (4.7%) | 0.106 |
| TP53 mutated | 5/54 (44.4%) | 7/86 (8.1%) | 1.000 |
| TP53 deleted and/or mutated | 8/54 (14.8%) | 19/86 (22.1%) | 0.336 |
| CD38 ≥30% | 24/54 (44.4%) | 8/86 (9.3%) | 3.9×10 ⁻⁶ |
| ZAP70 ≥20% | 18/51 (35.3%) | 20/83 (24.1%) | 0.231 |
| Clinical variables | | | |
| Age (years) | 70 (64-75) | 69 (61-77) | 0.949 |
| Male:female | 30:24 | 43:43 | 0.641 |
| ECOG PS >1 | 7/54 (13.0%) | 8/86 (9.3%) | 0.688 |
| Rai stage I-III | 16/54 (29.6%) | 16/86 (18.6%) | 0.192 |
| Lymph node involvement | 12/54 (22.2%) | 14/86 (16.3%) | 0.511 |
| Splenomegaly | 6/54 (11.1%) | 2/86 (2.3%) | 0.055 |
| Peripheral blood lymphocytes (×10 ⁹ /L) | 7.8 (5.8-16.7) | 10.5 (6.2-16.9) | 0.392 |
| Hb (g/dL) | 13.7 (12.9-14.8) | 13.9 (13.2-14.8) | 0.488 |
| Platelets (×10 ⁹ /L) | 218 (166-245) | 194 (168-236) | 0.266 |
| Bone marrow lymphocytes (%) | 40 (26-60) | 30 (15-50) | 0.006 |
| Diffuse bone marrow pattern | 10/54 (18.5%) | 6/84 (7.1%) | 0.078 |
| Beta-2-microglobulin (mg/L) | 2.4 (1.9-3.2) | 2.0 (1.7-2.7) | 0.020 |
| LDH (U/L) | 377 (309-436) | 335 (284-388) | 0.007 |
| Alkaline phosphatase (U/L) | 161 (127-193) | 149 (126-175) | 0.240 |
| Albumin (g/L) | 42 (39-45) | 43 (40-46) | 0.540 |
| Direct antiglobulin test | 8/44 (18.2%) | 3/43 (7.0%) | 0.211 |
| Serum monoclonal component | 8/86 (9.3%) | 15/54 (27.8%) | 0.008 |
| IgG (g/L) | 9.7 (7.9-11.5) | 9.6 (8.2-11.6) | 0.959 |
| IgA (g/L) | 1.5 (1.1-2.1) | 1.1 (0.7-1.6) | 0.009 |
| IgM (g/L) | 0.5 (0.3-0.9) | 0.5 (0.3-0.8) | 0.625 |
| CD4+ lymphocytes (×10 ⁹ /L) | 1.0 (0.6-2.0) | 0.8 (0.5-1.5) | 0.044 |

^a25th-75th percentiles are reported in parentheses for continuous variables; p, p value was calculated by χ^2 test with Yates correction and Fisher's exact test when appropriate for categorical variables and by Mann-Whitney test for continuous variables.

Supplementary Table S3. Biological and clinical variables at diagnosis of Binet A CLL predicting TFS by univariate analysis.^a

| | Events/N | Median (months) | 95% CI | 5-year risk (%) | 95% CI | p |
|------------------------------------|----------|-----------------|------------|-----------------|-----------|-----------------------|
| IGHV homology <98% | 21/95 | Not reached | — | 76.1 | 66.2-86.0 | 0.001 |
| IGHV homology ≥98% | 19/42 | 50.2 | 34.3-66.2 | 39.7 | 20.0-59.4 | |
| Number of FISH lesions ≤1 | 27/118 | Not reached | — | 73.7 | 63.9-83.5 | 5.2×10 ⁻⁴ |
| Number of FISH lesions >1 | 13/22 | 41.8 | 19.8-63.7 | 24.6 | 0-49.6 | |
| FISH stratification by Döhner | | | | | | 1.6×10 ⁻⁸ |
| del13q14 only | 5/60 | Not reached | — | 92.2 | 84.8-99.6 | |
| normal | 10/37 | Not reached | — | 64.6 | 44.7-84.5 | |
| +12 | 16/25 | 41.8 | 36.3-47.2 | 22.2 | 1.5-42.9 | |
| del11q22-q23 | 4/7 | 29.3 | 0-79.5 | 47.6 | 3.5-91.7 | |
| del17p13 | 11/5 | 40.0 | 0-81.7 | 39.3 | 1.5-77.1 | |
| FISH stratification | | | | | | 7.4×10 ⁻¹⁰ |
| del13q14 only | 5/60 | Not reached | — | 92.2 | 84.8-99.6 | |
| normal | 10/37 | Not reached | — | 64.6 | 44.7-84.5 | |
| del17p13/del11q22-q23/+12 | 25/43 | 40.0 | 25.2-54.7 | 28.8 | 10.8-46.8 | |
| TP53 wt | 35/128 | Not reached | — | 68.1 | 58.3-77.9 | 0.029 |
| TP53 mutated | 5/12 | 40.0 | 12.1-67.8 | 28.1 | 0-70.4 | |
| TP53 not inactivated | 34/125 | Not reached | — | 68.3 | 58.4-78.2 | 0.024 |
| TP53 deleted and/or mutated | 6/15 | 40.0 | 5.8-74.1 | 40.1 | 3.1-77.1 | |
| CD38 <30% | 23/108 | Not reached | — | 76.3 | 66.7-85.9 | 7.6×10 ⁻⁵ |
| CD38 ≥30% | 17/32 | 43.8 | 35.3-52.3 | 27.5 | 14.8-50.2 | |
| ZAP70 <20% | 22/96 | Not reached | — | 69.3 | 57.8-80.8 | 0.005 |
| ZAP70 ≥20% | 17/38 | 67.4 | 30.7-104.1 | 52.7 | 33.0-72.4 | |
| Age ≤70 year | 18/76 | Not reached | — | 74.9 | 63.6-86.2 | 0.010 |
| Age >70 year | 22/64 | 88.3 | 22.1-154.5 | 53.2 | 37.0-69.4 | |
| Rai | | | | | | 0.003 |
| 0 | 26/108 | Not reached | — | 71.6 | 61.3-81.9 | |
| I-III | 14/32 | 43.8 | 33.5-54.2 | 44.0 | 21.1-66.9 | |
| No splenomegaly | 35/132 | Not reached | — | 67.9 | 58.1-77.7 | 0.003 |
| Splenomegaly | 5/8 | 49.4 | 0-99.3 | 30.0 | 0-75.2 | |
| Lymphocytes ≤20×10 ⁹ /L | 25/119 | Not reached | — | 71.7 | 61.6-81.8 | 5.6×10 ⁻⁶ |
| Lymphocytes >20×10 ⁹ /L | 15/21 | 29.3 | 13.5-45.1 | 36.8 | 14.5-59.1 | |
| Hb >13 g/dL | 24/108 | Not reached | — | 73.2 | 62.7-83.7 | 0.002 |
| Hb <13 g/dL | 16/32 | 41.8 | 26.7-56.8 | 43.2 | 23.2-63.1 | |
| Platelets ≥150×10 ⁹ /L | 29/118 | Not reached | — | 70.3 | 60.2-80.4 | 0.002 |
| Platelets <150×10 ⁹ /L | 11/22 | 53.4 | 15.5-91.2 | 40.5 | 14.7-66.3 | |
| Bone marrow lymphocytes ≤50% | 23/107 | Not reached | — | 72.9 | 62.2-83.6 | 0.001 |
| Bone marrow lymphocytes >50% | 16/31 | 40.0 | 24.7-55.2 | 43.6 | 23.7-63.5 | |
| No diffuse bone marrow pattern | 29/122 | Not reached | — | 70.2 | 60.1-80.3 | 3.1×10 ⁻⁵ |
| Diffuse bone marrow pattern | 10/16 | 37.2 | 8.1-73.6 | 32.1 | 4.5-59.7 | |
| β-2-microglobulin ≤2.5 mg/L | 15/92 | Not reached | — | 75.8 | 64.5-87.1 | 1.4×10 ⁻⁴ |
| β-2-microglobulin >2.5 mg/L | 25/48 | 56.8 | 22.6-91.0 | 49.3 | 33.3-65.3 | |
| LDH ≤1×ULN | 30/120 | Not reached | — | 66.7 | 56.0-77.4 | 0.028 |
| LDH >1×ULN | 10/20 | 87.5 | 0-218.2 | 58.5 | 36.2-80.8 | |
| ALP ≤1×ULN | 24/107 | Not reached | — | 69.8 | 58.9-80.7 | 0.007 |
| ALP >1×ULN | 16/33 | 67.4 | 20.8-114.1 | 53.8 | 33.9-73.7 | |

^aEvents/N, number of events over number of patients; 5-year risk: 5-year treatment free survival calculated by Kaplan-Meier method; 95% CI: 95% confidence interval; p: p value calculated by log-rank; ULN; upper limit of normal.