Lymphoma and microenvironment

Valter Gattei, MD

Head Clinical and Experimental Oncology Unit
IRCCS
Aviano (PN)
CLL cells do need microenvironmental interactions to survive (proliferate)
CLL and microenvironment.....

....where....?

...lymph node / bone marrow.....
Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1

Jan A. Burger, Nobuhiro Tsukada, Meike Burger, Nathan J. Zvaifler, Marie Dell’Aquila and Thomas J. Kipps

Distinctive features of "nurselike" cells that differentiate in the context of chronic lymphocytic leukemia

Nobuhiro Tsukada, Jan A. Burger, Nathan J. Zvaifler and Thomas J. Kipps
...several axes...
CLL and microenvironment.....
Mantle cell lymphoma cells express high levels of CXCR4, CXCR5, and VLA-4 (CD49d): importance for interactions with the stromal microenvironment and specific targeting

Antonina V. Kurtova,¹ Archito T. Tamayo,² Richard J. Ford,² and Jan A. Burger¹

Departments of ¹Leukemia and ²Hematopathology, The University of Texas M. D. Anderson Cancer Center, Houston
VLA-4 (CD49d/CD29)

- heterodimeric integrin formed by non-covalent association of \( \alpha_4 \) (CD49d; 155kDa) and \( \beta_1 \) (CD29; 150kDa) subunits

- It functions as a matrix and cell receptor

- It is expressed on:
  - eosinophils
  - basophils
  - NK cells
  - monocytes
  - T cells
  - B cells
  - thymocytes
  - myeloma cells
LIGANDS OF CD49d

CD49d

Other Names
Integrin α4-chain, VLA-4α-chain.

VCAM-1
(Elices et al., 1990)

Fibronectin
(Wayne et al., 1989)

Emilin-1

VCAM-1/CD106

EMI Coiled-coil COL gC1q EMILIN-1
In vivo: CD49d ligands expression in CLL-involved area

VCAM-1

Fibronectin

Emilin-1
Emilin-1 (Elastin Microfibril Interface Located protein 1)

Emilin-1 in tonsil (marginal zone)

Courtesy of Paola Spessotto/Alberto Zamò
Negative prognostic marker in CLL

CD49d expression (% positive cells)

Variable expression in CLL

39% CD49d+

61% CD49d-

Negative prognostic marker in CLL
CD49d in CLL: loose ends

**Optimal cutoff point** (30%, 45%, other)?

**Independent prognostic relevance?**

**Prognostic relevance among flow cytometry-based markers?**
Worldwide multi-center meta-analysis on CD49d prognosis


**Shanafelt TD et al.** CD49d expression is an independent predictor of overall survival in patients with chronic lymphocytic leukaemia: A prognostic parameter with therapeutic potential. Br J Haematol. 2008;140:537-46

**Rossi D et al.** CD49d expression is an independent risk factor of progressive disease in early stage chronic lymphocytic leukemia. Haematologica. 2008;93:1575-9


**Cro L et al.** The clinical and biological features of a series of immunophenotypic variant of B-CLL. Eur J Haematol. 2010;85:120-9

**Shanafelt TD et al.** Vitamin D insufficiency and prognosis in chronic lymphocytic leukemia. Blood. 2011;117:1492-8

**Majid A et al.** CD49d is an independent prognostic marker that is associated with CXCR4 expression in CLL. Leuk Res. 2011;35:750-6

**Kurtova A et al.** The immunophenotype signature CD49d⁺CD38⁺ identifies chronic lymphocytic leukemia cases with a higher potential for migration beneath marrow stromal cell. Blood. 2009;114(abstract 356)
Worldwide multi-center meta-analysis on CD49d prognosis

Patient cohorts

- IPD retrieved for detailed evaluation n=3267
- IPD excluded for missing OS n=235
- potentially valid IPD n=3032
- IPD excluded for missing CD49d data n=60
- valid IPD n=2972

Cutoff searching (tr./val.)

- CD49d cut-off testing cohort n=1556 (published)
- CD49d cut-off validation cohort n=1416 (unpublished)

Multivariate analyses

- Pooled analysis n=2972

Flow cytometry markers
CD49d Is the Strongest Flow Cytometry–Based Predictor of Overall Survival in Chronic Lymphocytic Leukemia

Pietro Bulian, Tais D. Shanafelt, Chris Fegan, Antonella Zucchetto, Lilla Cro, Holger Nickel, Luca Baldini, Antonina V. Kurtova, Alessandra Ferrajoli, Jan A. Burger, Gianluca Gaidano, Giovanni Del Poeta, Chris Pepper, Davide Rossi, and Valter Garretti
Prognostic relevance among flow cytometry-based markers

Recursive partitioning

- Is CD49d pos?
  - No: n=1874 (63%), CP = 0.045
  - Yes: n=1098 (37%)

- Is CD38 pos?
  - No: n=1582 (53%)
  - Yes: n=292 (10%), CP < 0.01

- Is ZAP_70 pos?
  - No: n=1256 (42%)
  - Yes: n=326 (11%)

- Is CD38 pos?
  - No: n=198 (7%)
  - Yes: n=198 (7%)

- Is ZAP_70 pos?
  - No: n=288 (10%)
  - Yes: n=181 (6%)

- Is CD38 pos?
  - No: n=184 (6%)
  - Yes: n=445 (15%)
### Independent prognostic relevance (30% cutoff pooled series)

**Table 2. Multivariate Cox regression analysis of OS**

<table>
<thead>
<tr>
<th>CD49d included</th>
<th></th>
<th></th>
<th></th>
<th>CD49d excluded</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>final reduced model</td>
<td>initial full model</td>
<td>univariate model</td>
<td>final reduced model</td>
<td>initial full model</td>
<td>univariate model</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HR</td>
<td>95% CI</td>
<td>p</td>
<td>HR</td>
<td>95% CI</td>
<td>p</td>
<td>HR</td>
</tr>
<tr>
<td>age &gt;65years</td>
<td>3.25</td>
<td>2.18 - 4.84</td>
<td>6 x10^-9</td>
<td>3.03</td>
<td>2.00 - 4.57</td>
<td>1x10^-7</td>
<td>2.75</td>
</tr>
<tr>
<td>UM IGHV</td>
<td>2.48</td>
<td>1.68 - 3.66</td>
<td>5 x10^-5</td>
<td>1.99</td>
<td>1.29 - 3.07</td>
<td>0.0020</td>
<td>2.64</td>
</tr>
<tr>
<td>CD49d≥30%</td>
<td>2.26</td>
<td>1.57 - 3.25</td>
<td>1 x10^-5</td>
<td>2.01</td>
<td>1.36 - 2.97</td>
<td>0.0005</td>
<td>2.31</td>
</tr>
<tr>
<td>Del 17p</td>
<td>2.13</td>
<td>1.37 - 3.32</td>
<td>0.0008</td>
<td>2.22</td>
<td>1.41 - 3.50</td>
<td>0.0006</td>
<td>2.90</td>
</tr>
<tr>
<td>gender (M)</td>
<td>1.83</td>
<td>1.24 - 2.69</td>
<td>0.0022</td>
<td>1.73</td>
<td>1.18 - 2.56</td>
<td>0.0055</td>
<td>1.53</td>
</tr>
<tr>
<td>ALC&gt;15x10^9/L</td>
<td>1.66</td>
<td>1.11 - 2.48</td>
<td>0.0133</td>
<td>1.62</td>
<td>1.08 - 2.43</td>
<td>0.0202</td>
<td>1.55</td>
</tr>
<tr>
<td>ZAP-70</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.45</td>
<td>0.95 - 2.21</td>
<td>0.0864</td>
<td>2.27</td>
</tr>
<tr>
<td>β2M above ULN</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.26</td>
<td>0.80 - 1.97</td>
<td>0.3217</td>
<td>2.23</td>
</tr>
<tr>
<td>CD38</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.21</td>
<td>0.82 - 1.78</td>
<td>0.3447</td>
<td>1.98</td>
</tr>
<tr>
<td>Del 11q</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.19</td>
<td>0.70 - 2.04</td>
<td>0.5244</td>
<td>1.35</td>
</tr>
</tbody>
</table>

**Table Notes:**
- HR: Hazard Ratio
- 95% CI: 95% Confidence Interval
- p: p-value
CD49d Is the Strongest Flow Cytometry–Based Predictor of Overall Survival in Chronic Lymphocytic Leukemia

Pietro Bulian, Taie D. Shanafelt, Chris Fegan, Antonella Zucchetto, Lilia Cro, Holger Nüchel, Luca Baldini, Antonina V. Kurtova, Alessandra Ferrajoli, Ian A. Burger, Gianluca Gaidano, Giovanni Del Poeta, Chris Pepper, Davide Rossi, and Valter Gattei

clinical impact

IGHV

CD49d

Genetics Del TP53

NOTCH1

BIRC3

TP53

SF3B1

trisomy12
del11

ZAP-70

CD38
CD49d is the strongest Flow Cytometry–Based Predictor of Overall Survival in Chronic Lymphocytic Leukemia

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CD49d Prevails over the Novel Recurrent Mutations As Independent Prognosticator of Overall Survival in Chronic Lymphocytic Leukemia

Michele Dal Bo1, Pietro Bulian1, Riccardo Bomben1, Antonella Zucchetto1, Francesca Rossi1, Federico Pozzo1, Tamara Bittolo1, Paola Nanni1, Ilaria Cattarossi1, Eva Zaina1, Hillary Chivio1, Massimo Degani1, Francesco Zaja2, Gabriele Pozzato2, Annalisa Chiarenza2, Francesco Di Raimondo4, Giovanni Del Poeta5, Davide Rossi6, Gianluca Gaidano6 and Valter Gattel1

1Clinical and Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico, I.R.C.C.S., Aviano, Italy; 2 Centro Trapianti e Terapie Cellulare “Carlo Melzi” DISM, Azienda Ospedaliera Universitaria S. Maria Misericordia, Udine, Italy; 3 Department of Internal Medicine and Haematology, Maggiore General Hospital, University of Trieste, Trieste, Italy; 4 Division of Hematology, Ferrarotto Hospital, Catania, Italy; 5 Division of Hematology, S. Eugenio Hospital and University of Tor Vergata, Roma, Italy; 6 Division of Hematology – Department of Translational Medicine – Amedeo Avogadro University of Eastern Piedmont, Novara, Italy

778 fully characterized CLL
CASE COHORT

The study and included 778 unselected CLL (422 untreated and 356 treated cases), with CLL according to the current guidelines. The median age at the diagnosis was 67 years (range: 33-91 years).

The distribution of clinical stages at diagnosis, according to Rai staging system, was as follows: 389/778 (50%) patients, stage 0, 241/778 (31%) patients, stage I, 99/778 (12.7%) patients, stage II, 14/778 (1.8%) patients, stage III, 35/778 (4.5%) patients, stage IV.

An unmutated IGHV status was detected in 262/778 (33.7%) cases.

According to the hierarchical stratification of karyotype abnormalities: 308/778 (39.6%) cases were 13q-, 103/778 (13.2%) cases were +12, 64/778 (8.2%) cases were 11q-, 58/778 (7.5%) were 17p-, 45/778 (5.8%) cases showed a deletion of the BIRC3 locus. Of note, in all the 45 cases the deletion of the BIRC3 locus was concomitant to the canonical 11q deletion encompassing the ATM locus.
**CASE COHORT**

When CD49d expression was evaluated by flow cytometry, 229/778 (29.4%) cases were defined CD49dhigh using the 30% cut-off.

*BIRC3* mutations were detected in 20/778 (2.6%) cases.

*NOTCH1* mutations were detected in 81/778 (10.4%) cases.

*SF3B1* mutations were detected in 54/778 (6.9%) cases.

*TP53* mutations were detected in 61/778 (7.8%) cases.
In vivo: VCAM-1 expression in CLL-involved area of BMB

VCAM-1 staining

CD38-CD49d-CCL3- CLL

CD38+CD49d+CCL3+ CLL
Integrated mutational and cytogenetic analysis identifies new prognostic subgroups in chronic lymphocytic leukemia

Davide Rossi, Silvia Rasi, Valeria Spina, Alessio Bruscaggin, Sara Monti, Carmela Ciardullo, Clara Deambrogi, Hossein Khialianfar, Roberto Serra, Francesco Bertoni, Francesco Forconi, Luca Laurenti, Roberto Marasca, Michele Dal-Bo, Francesco Maria Rossi, Pietro Bulian, Josep Nomdedeu, Giovanni Del Poeta, Valer Gattei, Laura Pasqualucci, Raul Rababan, Robin Foà, Riccardo Dalla-Favera, and Gianluca Gaidano

Division of Hematology, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy; Department of Biomedical Informatics and Center for Computational Biology and Bioinformatics, Columbia University, New York, NY; Laboratory of Medical Informatics, Department of Translational Medicine, Amedeo Avogadro University of Eastern Piedmont, Novara, Italy; Lymphoma and Genomics Research Program, IOR-Institute of Oncology Research and Lymphoma Unit, Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; Cancer Sciences Unit, CRUK Clinical Centre, University of Southampton, Southampton, United Kingdom; Division of Hematology, University of Siena, Siena, Italy; Institute of Hematology, Catholic University of the Sacred Heart, Rome, Italy; Division of Hematology, Department of Oncology and Hematology, University of Modena and Reggio Emilia, Modena, Italy; Clinical and Experimental Oncology-Hematology, Centro di Riferimento Oncologico, Aviano, Italy; Department of Hematology and Laboratory Medicine, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain; Department of Hematology, Tor Vergata University, Rome, Italy; Institute for Cancer Genetics and the Herbert Irving Comprehensive Cancer Center, Columbia University, New York, NY; Department of Pathology & Cell Biology, Columbia University, New York, NY; Institute of Hematology, Department of Hematology and Clinical Immunology, University of Pavia, Pavia, Italy; Division of Hematology, Department of Cellular Biotechnologies and Hematology, Sapienza University, Rome, Italy; and Department of Genetics & Development, Columbia University, New York, NY.

Figure 2. Kaplan-Meier estimates of OS and treatment-free survival according to the integrated mutational and cytogenetic model in the training series. (A) OS. (B) Probability of progressive disease requiring treatment according to IWCLL-NCI guidelines as indicated by treatment-free interval. Cases harboring TP53 and/or BIRC3 disruption (TP53 DIS/BIRC3 DIS) independent of cooccurring genetic lesions are represented by the red line. Patients harboring NOTCH1 mutations (NOTCH1 M) and/or SF3B1 mutations (SF3B1 M) and/or del11q22-q23 in the absence of TP53 and BIRC3 disruption are represented by the yellow line. Patients harboring +12 in the absence of the TP53 disruption, BIRC3 disruption, NOTCH1 mutations, SF3B1 mutations, and del11q22-q23 and patients wild-type for all genetic lesions (normal) are represented by the green line. Cases harboring del13q14 as the sole genetic lesion are represented by the blue line. nm indicates not reached.
The integrated mutational/cytogenetic model in the 778 cohort

<table>
<thead>
<tr>
<th>Survival probability (%)</th>
<th>Time (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>80</td>
<td>50</td>
</tr>
<tr>
<td>60</td>
<td>100</td>
</tr>
<tr>
<td>40</td>
<td>150</td>
</tr>
<tr>
<td>20</td>
<td>200</td>
</tr>
<tr>
<td>0</td>
<td>250</td>
</tr>
</tbody>
</table>

- **13q-**
  - vs. normal karyotype/ +12: p = 0.1240
  - vs. NOTCH1 mutations/ SF3B1 mutations / 11q-: p < 0.0001
  - vs. BIRC3 disruption/ TP53 disruption: p < 0.0001

- **normal karyotype/ +12**
  - vs. NOTCH1 mutations/ SF3B1 mutations / 11q-: p < 0.0001
  - vs. BIRC3 disruption/ TP53 disruption: p < 0.0001

- **NOTCH1 mutations/ SF3B1 mutations / 11q-**
  - vs. BIRC3 disruption/ TP53 disruption: p = 0.1156
**Table 1. Multivariate Cox regression analysis of OS in the whole CLL cohort (778 cases)**

<table>
<thead>
<tr>
<th></th>
<th>Sample size</th>
<th>HR (95% CI)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD49&lt;sup&gt;high&lt;/sup&gt;</td>
<td>778</td>
<td>1.88 (1.36-2.60)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>age ≥65 years</td>
<td></td>
<td>3.90 (2.68-5.67)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Rai stage I or higher</td>
<td></td>
<td>1.80 (1.30-2.51)</td>
<td>0.0005</td>
</tr>
<tr>
<td>UM <em>IGHV</em> status</td>
<td></td>
<td>1.84 (1.31-2.60)</td>
<td>0.0005</td>
</tr>
<tr>
<td>11q-</td>
<td></td>
<td>2.21 (1.39-3.51)</td>
<td>0.0008</td>
</tr>
<tr>
<td>NOTCH1 mutations</td>
<td></td>
<td>1.79 (1.17-2.73)</td>
<td>0.0068</td>
</tr>
<tr>
<td>TP53 disruption</td>
<td></td>
<td>3.65 (2.51-5.30)</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Multivariate Cox regression analysis of OS was performed by including the following covariates: CD49d, age, *IGHV* status, 13q-, +12, 11q-, *BIRC3* disruption, *SF3B1* mutations, *NOTCH1* mutations, *TP53* disruption For each variable, the selected cut-off points or categories are indicated. OS, Overall Survival.

Based on the final model after stepwise selection of covariates. HR, hazard ratio; CI, confidence interval.
CD49 and IGHV status in the integrated mutational/cytogenetic categories
Inclusion of CD49 and IGHV status in the integrated mutational/cytogenetic model

Group 1
CD49d<sub>low</sub> with
13q- / normal karyotype / +12 / NOTCH1 mutations / SF3B1 mutations / 11q-

Group 2
CD49d<sub>high</sub> with
13q- / normal karyotype / +12

Group 3
CD49d<sub>high</sub> with
NOTCH1 mutations / SF3B1 mutations / 11q-

or M IGHV with
BIRCl3 disruption / TP53 disruption

Group 4
UM IGHV with
BIRCl3 disruption / TP53 disruption
CD49d Is the Strongest Flow Cytometry–Based Predictor of Overall Survival in Chronic Lymphocytic Leukemia

Pietro Bulian, Tait D. Shanafelt, Chris Fegan, Antonella Zucchetti, Lilla Cro, Holger Nickel, Luca Baldini, Antonina V. Kurtova, Alessandra Ferrajoli, Jan A. Burger, Gianluca Gaidano, Giovanni Del Poeta, Chris Pepper, Davide Rossi, and Valter Gattei

clinical impact

IGHV

CD49d

Genetics Del TP53

NOTCH1

BIRC3

TP53

SF3B1

trisomy12
del11

ZAP-70

CD38
CD49d Is the Strongest Flow Cytometry–Based Predictor of Overall Survival in Chronic Lymphocytic Leukemia

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IGHV

CD49d

Genetics del/mut TP53

NOTCH1

ZAP-70

CD38

trisomy12
del11
CD38

CD49d

“humoral” link

“physical” link

CD38

CD49d
CD49d & CD38 functionally linked in a humoral network

CD68+ cell infiltration in CLL-involved area of BMB

CD38-CD49d-CCL3- CLL

CD38+CD49d+CCL3+ CLL

CD68 staining
CCL3/CCL4 in plasma and prognosis (Sivina et al Blood, 2011)

CD68+ macrophages and prognosis ??

Tumor-Associated Macrophages and Survival in Classic Hodgkin’s Lymphoma

Christian Steidl, M.D., Tang Lee, M.Sc., Sohrab P. Shah, Ph.D., Pedro Farinha, M.D., Guangming Han, M.D., Tarun Nayar, M.Sc., Allen Delaney, Ph.D., Steven J. Jones, Ph.D., Javeed Iqbal, Ph.D., Dennis D. Weisenburger, M.D., Martin A. Bast, B.S., Andreas Rosenwald, M.D., Hans-Konrad Muller-Hermelink, M.D., Lisa M. Rimsza, M.D., Elias Campo, M.D., Ph.D., Jan Delabie, M.D., Ph.D., Rita M. Braziel, M.D., James R. Cook, M.D., Ray R. Tubbs, D.O., Elaine S. Jaffe, M.D., Georg Lenz, M.D., Joseph M. Connors, M.D., Louis M. Staudt, M.D., Ph.D., Wing C. Chan, M.D., and Randy D. Gascoyne, M.D.
CD38

CD49d

"physical" link

"humoral" link

CD38
CD49d and regulation of activation

ORIGINAL ARTICLE
The CD49d/CD29 complex is physically and functionally associated with CD38 in B-cell chronic lymphocytic leukemia cells

A Zucchetto¹, T Vaisitti², D Benedetti¹, E Tissino¹, V Bertagnolo³, D Rossi⁴, R Bomben¹, M Dal Bo¹, MI Del Principe⁵, A Gorgone⁶, G Pozzato⁷, G Gaidano⁴, G Del Poeta⁵, F Malavasi⁸, S Deaglio²,¹⁰ and V Gattein¹,¹⁰
CD49d and CD38 are physically associated

a. Capping experiments and confocal microscopy analysis

mAb-mediated capping (anti-CD49d)

counterstaining (anti-CD38)

merge

b. Immunoprecipitation experiments

(Zucchetto et al, Leukemia 2012; Buggins et al, Br J Haematol 2012)
CD49d-CD38 association is maintained after polarization of CD49d to its natural ligands.
CD38 favors CLL cells adhesion and spreading onto CD49d substrates

CD49d+ CD38- CLL

CD49d+ CD38+ CLL

![Graph showing mean number of adherent cells/field for CD49d+CD38- CLL and CD49d+CD38+ CLL with p=0.0001]
BCR-mediated

inside-out mechanism of CD49d activation

CD38-mediated

IGHV → CD49d

CD38
inside-out mechanism of CD49d activation

Woyach J.A., Blood 2012
HUTS21 (anti-CD29)
LDV (VLA-4 ligand)
The HUTS-21/LDV approach is used to detect the active state of VLA-4

**Receptor Occupancy**

\[
RO = \frac{1}{1 + 10^{(\log EC_{50} - \log[LDV])}}
\]

- RO=0.0 (0%)
- RO=1.0 (100%)

**EC\textsubscript{50}:** ligand’s concentration which induces a response halfway between the baseline and maximum.

VLA-4 receptor occupancy would increase from \(~0.24\) to \(~0.76\) after the affinity change.

- **EC\textsubscript{50}**: ligand’s concentration which induces a response halfway between the baseline and maximum.

---

Alexandre Chigaev et al. J. Biol. Chem. 2009;284:14337-14346
BCR STIMULATION IS ABLE TO INCREASE VLA-4 RECEPTOR OCCUPANCY IN CLL

RO = 0.60
(BCR stimulation)

RO = 0.30
(control)

N=18
inside-out mechanism of CD49d activation

Woyach J.A., Blood 2012
Ibrutinib and CLL microenvironment

Irreversible BTK inhibitor, that fits tightly into the ATP binding pocket

Blood lymphocytosis was generally noted by day 7 (in 78% of the patients); it peaked at a median of 4 weeks and then slowly declined

Lymphocytosis occurred concomitantly with a notable reduction in lymph-node size

Ibrutinib and CLL microenvironment

Treatment with Ibrutinib Inhibits BTK- and VLA-4-Dependent Adhesion of Chronic Lymphocytic Leukemia Cells In Vivo

Sarah E.M. Herman¹, Rashida Z. Mustafa¹, Jade Jones¹,², Deanna H. Wong¹, Mohammed Farooqui¹, and Adrian Wiestner¹

LYMPHOID NEOPLASIA

Brief report

The clinically active BTK inhibitor PCI-32765 targets B-cell receptor– and chemokine-controlled adhesion and migration in chronic lymphocytic leukemia

Martin F. M. de Rooij,¹ Annemieke Kuil,¹ Christian R. Geest,² Eric Eldering,² Betty Y. Chang,³ Joseph J. Buggy,³
*Steven T. Pals,¹ and *Marcel Spaargaren¹

Departments of ¹Pathology and ²Experimental Immunology, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands; and ³Pharmacys Inc, Sunnyvale, CA

Inside-out VLA-4 activation NOT investigated
CD49d and regulation of activation

Does inhibition of the BCR signaling by IBRUTINIB exposure affect VLA-4 activation in CLL cells?
CD49d+ CLL have a reduced IB-induced redistribution lymphocytosis + reduced lymph node shrinkage (to be confirmed)
Trisomy 12 is associated with an abbreviated redistribution lymphocytosis during treatment with the BTK inhibitor ibrutinib in patients with chronic lymphocytic leukaemia

LYMPHOID NEOPLASIA
CD49d is overexpressed by trisomy 12 chronic lymphocytic leukemia cells: evidence for a methylation-dependent regulation mechanism
inside-out mechanism of CD49d activation
CD49d and regulation of activation

B-cell receptor (BCR) engagement leads to activation integrins

- LFA-1 (VLA-4)
- VLA-4

**Inhibitors:**
- idelalisib
- fosfamatinib
- ibrutinib

**Signaling Pathways:**
- Lyn and Syk
- PI3K
- PLCγ2
- Rac2
- Rap1
- Ca²⁺
- IP₃
- DAG
- PKC
CD49d

clinical impact

CD49d as prognostic marker

regulation of activation (impact on novel therapies)

CD49d as predictive marker