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## IL1RAP expression as a measure of leukemic stem cell burden at diagnosis of chronic myeloid leukemia predicts therapy outcome.

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Published in:  
Leukemia

DOI:  
[10.1038/leu.2015.135](https://doi.org/10.1038/leu.2015.135)

2015

[Link to publication](#)

### Citation for published version (APA):

Landberg, N., Hansen, N., Askmyr, M., Ågerstam, H., Lassen, C., Rissler, M., Hansen, H. H., Mustjoki, S., Järås, M., Richter, J., & Fioretos, T. (2015). IL1RAP expression as a measure of leukemic stem cell burden at diagnosis of chronic myeloid leukemia predicts therapy outcome. *Leukemia*, 30(1), 255-258. <https://doi.org/10.1038/leu.2015.135>

Total number of authors:  
11

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1 **IL1RAP expression as a measure of leukemic stem cell burden at diagnosis of**  
2 **chronic myeloid leukemia predicts therapy outcome**

3

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17

18 **Running title:** IL1RAP as a prognostic marker in CML

19

20 **Key words:** chronic myeloid leukemia, leukemic stem cells, prognosis, tyrosine kinase

21 inhibitors

22 **Conflicts of interest**

23 M.J. and T.F. are cofounders and have equity ownership in Cantargia AB (Ideon Medical  
24 Village, Lund, Sweden) formed with Lund University Bioscience AB. J.R. has stock  
25 options in Cantargia AB and has received honoraria from Novartis and Bristol-Myers  
26 Squibb. S.M. has received research funding from Novartis, Bristol-Myers and Squibb and  
27 Pfizer and honoraria from Novartis and Bristol-Myers and Squibb. The remaining  
28 authors declare no competing financial interests.

29

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46 **LETTER TO THE EDITOR**

47 Chronic myeloid leukemia (CML) is characterized by the *BCR/ABL1* fusion gene formed  
48 by a t(9;22) chromosomal translocation. The t(9;22) is thought to arise in a  
49 hematopoietic stem cell (HSC) creating a hierarchically arranged leukemia with a subset  
50 of cells in the CD34<sup>+</sup>CD38<sup>low</sup> fraction comprising the primitive leukemia initiating cells,  
51 also termed leukemic stem cells (LSCs).<sup>1,2</sup> The treatment of CML was greatly improved  
52 with the introduction of tyrosine kinase inhibitors (TKIs) more than a decade ago, but  
53 not all patients respond optimally to initial treatment and even after obtaining a deep  
54 molecular remission, discontinuation of treatment is often followed by relapse.<sup>3-5</sup>

55           The LSC-burden of CML patients at diagnosis could potentially provide an  
56 important disease variable for predicting response to TKI treatment. We previously  
57 estimated the LSC-burden at diagnosis of CML by quantification of primitive  
58 CD34<sup>+</sup>CD38<sup>low</sup> *BCR/ABL1* positive cells, which was predictive of response to TKI  
59 therapy.<sup>6</sup> However, this procedure would be challenging to implement clinically because  
60 it requires both sorting of CD34<sup>+</sup>CD38<sup>low</sup> CML cells and fluorescent in situ hybridization  
61 (FISH) analysis to enumerate *BCR/ABL1* positive cells. Here, we aimed at establishing a  
62 flow cytometry-based protocol to estimate the LSC-burden at diagnosis by exploring the  
63 expression of cell surface markers reported to be upregulated on primitive CML  
64 CD34<sup>+</sup>CD38<sup>low</sup> cells.

65           Several markers have recently been proposed to be upregulated on  
66 candidate CML stem cells, including IL1RAP,<sup>7</sup> CD25 (IL2RA),<sup>8</sup> CD26 (DPP4),<sup>8</sup> CD123  
67 (IL3RA),<sup>9</sup> , CD117(KIT),<sup>10</sup> and ST2 (IL1RL1 or IL33R).<sup>11</sup> Of these markers, only CD26 and  
68 IL1RAP, a co-receptor of the interleukin 1 receptor (IL1R1) with unknown function in  
69 normal and malignant hematopoiesis, have so far been proven to separate *BCR/ABL1*  
70 positive from negative cells within the CD34<sup>+</sup>CD38<sup>low</sup> fraction.<sup>7,8</sup> Here, we first used a

71 staining protocol to simultaneously analyze the expression of these seven markers in  
72 bone marrow aspirates from five CML patients and two normal bone marrows (NBM).  
73 The LSC-containing CML CD34<sup>+</sup>CD38<sup>low</sup> compartment was defined as shown in Figure  
74 1a. We found that IL1RAP, CD25 and CD26 were distinctly upregulated on primitive CML  
75 cells relative to corresponding cells in NBM, whereas the other markers showed a  
76 variable expression pattern or were expressed also on CD34<sup>+</sup>CD38<sup>low</sup> NBM cells (Figure  
77 1b and Supplemental Table 1). CD123, CD117 and IL1R1 were all expressed on the  
78 immature CML CD34<sup>+</sup>CD38<sup>low</sup> cells but showed expression also on the normal HSC. In  
79 contrast to a recent study reporting low ST2 expression on CML CD34<sup>+</sup>CD38<sup>low</sup> cells,<sup>11</sup>  
80 this marker could only be detected in one of five CML patients using the same antibody,  
81 possibly because of a lower sensitivity of our flow cytometry protocol. Thus IL1RAP,  
82 CD25 and CD26 were specifically expressed on CML CD34<sup>+</sup>CD38<sup>low</sup> cells compared to  
83 corresponding normal cells.

84           To study the expression of LSC markers in more detail, IL1RAP, CD25 and  
85 CD123 were chosen for further analysis using an optimized panel of antibodies. As CD25  
86 and CD26 displayed similar expression patterns in primitive CML cells (Figure 1c),  
87 consistent with previous studies,<sup>8</sup> we included only CD25 in the staining protocol.  
88 Instead, we selected CD123 since antibodies targeting this receptor currently are being  
89 evaluated as a therapy in CML.<sup>9</sup> These three markers were analyzed in a cohort of 21  
90 CML patients included in the NordCML006 study in which patients were randomized to  
91 receive treatment either with imatinib or dasatinib.<sup>12</sup> A summary of patient data is  
92 provided in Supplementary Table 2. Using the optimized protocol, we obtained a clear  
93 separation of both IL1RAP positive and CD25 positive cells within the CML  
94 CD34<sup>+</sup>CD38<sup>low</sup> compartment, whereas CD123 expression failed to divide cells into  
95 distinct positive and negative cell populations (Figure 2a). In NBM, we did not observe

96 expression of IL1RAP or CD25 in the CD34<sup>+</sup>CD38<sup>low</sup> population, while CD123 was  
97 weakly expressed (Supplementary Figure 1). When analyzing co-expression of IL1RAP  
98 and CD25 in the CML CD34<sup>+</sup>CD38<sup>low</sup> compartment, we found one population co-  
99 expressing IL1RAP and CD25 and one expressing only IL1RAP. No cells were found to  
100 express only CD25 (Figure 2b). Although the cell fractions expressing IL1RAP and CD25  
101 showed a significant correlation (Spearman's Rho 0.8558, p<0.0001), IL1RAP was  
102 present on a consistently larger fraction of CML CD34<sup>+</sup>CD38<sup>low</sup> cells (mean of difference  
103 25.62%, CI 95% 15.81-35.44, p<0.0001).

104 To further delineate the difference between cells expressing IL1RAP and  
105 CD25, we correlated the flow cytometry data on IL1RAP and CD25 expression from the  
106 current study to previously reported *BCR/ABL1* FISH data on the CML CD34<sup>+</sup>CD38<sup>low</sup>  
107 population from the same patients.<sup>6</sup> IL1RAP expression showed a strong correlation  
108 with *BCR/ABL1* positivity in the CML CD34<sup>+</sup>CD38<sup>low</sup> fraction (r=0.8078, p<0.0001), with  
109 a slope of regression line close to 1 (Y=0.8876X-6.6)(Figure 2c). This indicates that  
110 almost all CD34<sup>+</sup>CD38<sup>low</sup> *BCR/ABL1* positive cells express IL1RAP and that the  
111 *BCR/ABL1* negative cells lack IL1RAP. Only a weak correlation was seen between  
112 *BCR/ABL1* positive cells and CD25 expression (r=0.4532, p=0.0391) with a slope of  
113 regression of 0.36 (Y=0.3584X+2.6), indicating that CD34<sup>+</sup>CD38<sup>low</sup> *BCR/ABL1* positive  
114 cells often lack CD25 expression (Figures 2d). We then used a previously described  
115 Flow-FISH sorting technique to sort CD34<sup>+</sup>CD38<sup>low</sup> cells according to CD25 expression  
116 and evaluated the fraction *BCR/ABL1* positive cells in four CML patients. We found that  
117 of the CD25 expressing cells, a mean of 98% were *BCR/ABL1* positive cells (ranging  
118 between 94-100%), whereas the CD25 negative population of the same patients still  
119 contained a mean of 85% *BCR/ABL1* positive cells (range between 46-99%). Consistent  
120 with our previous findings,<sup>7</sup> sorted IL1RAP positive CD34<sup>+</sup>CD38<sup>low</sup> cells contained a

121 great majority (99%) of *BCR/ABL1* positive cells (Supplementary Figure 2), whereas  
122 IL1RAP negative cells were almost all *BCR/ABL1* negative (5%). Taken together, these  
123 data show that IL1RAP is a marker for *BCR/ABL1* positive cells in the CML CD34<sup>+</sup>CD38<sup>low</sup>  
124 fraction, whereas CD25 is only expressed on a subfraction of *BCR/ABL1* positive cells.

125           The response of CML to TKI treatment is currently monitored by  
126 cytogenetic analyses and real time quantitative reverse polymerase chain reaction (RQ-  
127 PCR) analysis of *BCR/ABL1* transcript levels.<sup>4</sup> However, so far no easily implementable  
128 laboratory-based method is available to estimate the LSC-burden at diagnosis as a  
129 possible predictor of treatment response. We therefore investigated whether IL1RAP or  
130 CD25 expression in the CML CD34<sup>+</sup>CD38<sup>low</sup> compartment, as a measure of the LSC-  
131 burden at diagnosis, could provide clinically relevant prognostic information. In our  
132 previous study, the presence of ≥80% *BCR/ABL1* positive cells by FISH in the  
133 CD34<sup>+</sup>CD38<sup>low</sup> fraction at diagnosis of CML was used to define a group of patients with  
134 'high' LSC-burden, whereas <80% were designated as 'low' LSC-burden.<sup>6</sup> Based on  
135 IL1RAP expression, we used a similar definition, i.e. patients were classified as  
136 IL1RAP<sup>low</sup> (n=13, defined as <80% of the CD34<sup>+</sup>CD38<sup>low</sup> cells expressing IL1RAP) or  
137 IL1RAP<sup>high</sup> (n=8, ≥80% of CD34<sup>+</sup>CD38<sup>low</sup> cells expressing IL1RAP). Following this  
138 subdivision, we investigated if the two groups differed in the probability of achieving  
139 optimal response as defined by the European Leukemia Net (ELN) guidelines, following  
140 TKI treatment (Supplementary Table 3).<sup>4</sup> All IL1RAP<sup>low</sup> and 7 of 8 IL1RAP<sup>high</sup> patients  
141 achieved *BCR/ABL1* transcript levels ≤10% within 3 months. However, IL1RAP<sup>low</sup>  
142 patients had a higher chance of reaching transcript levels <1% after 6 months  
143 (p=0.0475), a definition of optimal response according to ELN.<sup>4</sup> Since this cohort  
144 consisted of mainly optimal responders at 3 months, we looked at even deeper levels of  
145 molecular and cytogenetic response, previously shown to predict long-term outcome at

146 this time point.<sup>13,14</sup> Interestingly, IL1RAP<sup>low</sup> patients (13 out of a total of 21) had a higher  
147 chance of achieving complete cytogenetic response (CCyR) (p=0.0009) and major  
148 molecular response (MMR; *BCR/ABL1* transcript levels  $\leq 0.1\%$ ) after 3 months (p=0.023)  
149 (Supplementary Table 3). No significant correlations were seen between IL1RAP  
150 expression and Sokal or Hasford risk scores. Altogether, this suggests that IL1RAP  
151 expression as a measure of LSC-burden at diagnosis predicts cytogenetic and molecular  
152 response to TKI treatment although larger prospective studies are needed before such  
153 measurement can be implemented clinically.

154           Previously, light scattering properties together with CD34 and CD45  
155 expression have been shown to differentiate between *BCR/ABL1* positive and negative  
156 CML CD34<sup>+</sup>CD38<sup>low</sup> cells.<sup>15</sup> Using that protocol, it was shown that the LSC-burden at  
157 diagnosis could predict CCyR after 1 year of treatment as well as MMR after 1.5 years.  
158 No correlations were made to response after 3 months of therapy. Other investigators  
159 have used CD26 as a marker for primitive CML cells and showed a correlation with  
160 leukocyte counts at diagnosis but not with Sokal or Hasford risk scores, possible  
161 association to cytogenetic and molecular response were not reported.<sup>8</sup> In contrast to our  
162 results, the authors could not detect overexpression of IL1RAP on primitive CML cells in  
163 all patients. Most likely, the difference in the two studies is a result of different  
164 sensitivities of the two assays to detect IL1RAP expression. All patients in our study  
165 clearly expressed IL1RAP; even in a patient in which FISH analysis of sorted  
166 CD34<sup>+</sup>CD38<sup>low</sup> cells revealed <1% *BCR/ABL1* positive cells, a fraction of cells expressed  
167 IL1RAP (patient no 10, Supplementary Table 2).

168           In conclusion, we identified IL1RAP, CD25 and CD26 as the most specific  
169 markers for primitive CML cells relative to corresponding normal cells and demonstrate  
170 that the percentage of IL1RAP expressing cells within the CD34<sup>+</sup>CD38<sup>low</sup> compartment



171 can be used to predict the response to TKI treatment. The described flow cytometry-  
172 based protocol may become a valuable prognostic tool in the management of CML  
173 patients.

174

175 Supplementary information is available at Leukemia's website

176

177 **Acknowledgements**

178 We thank all investigators of the NordCML006 study for sharing samples and clinical  
179 data. This work was supported by the Swedish Cancer Society, the Swedish Children's  
180 Cancer Foundation, the Swedish Research Council, the Inga-Britt and Arne Lundberg  
181 Foundation, the Gunnar Nilsson Cancer Foundation, the Medical Faculty of Lund  
182 University, the strategic research program BioCARE, the Finnish Cancer Institute, and  
183 the Academy of Finland.

184 **References**

- 185 1 Ren R. Mechanisms of BCR–ABL in the pathogenesis of chronic myelogenous  
186 leukaemia. *Nat Rev Cancer* 2005; **5**: 172–183.
- 187 2 Sloma I, Jiang X, Eaves AC, Eaves CJ. Insights into the stem cells of chronic myeloid  
188 leukemia. *Leukemia* 2010; **24**: 1823–1833.
- 189 3 Deininger M, Buchdunger E, Druker BJ. The development of imatinib as a  
190 therapeutic agent for chronic myeloid leukemia. *Blood* 2005; **105**: 2640–2653.
- 191 4 Baccarani M, Deininger MW, Rosti G, Hochhaus A, Soverini S, Apperley JF *et al.*  
192 European LeukemiaNet recommendations for the management of chronic myeloid  
193 leukemia: 2013. *Blood* 2013; **122**: 872–884.
- 194 5 Mahon FX, Etienne G. Deep Molecular Response in Chronic Myeloid Leukemia: The  
195 New Goal of Therapy? *Clinical Cancer Research* 2014; **20**: 310–322.
- 196 6 Mustjoki S, Richter J, Barbany G, Ehrencrona H, Fioretos T, Gedde-Dahl T *et al.*  
197 Impact of malignant stem cell burden on therapy outcome in newly diagnosed  
198 chronic myeloid leukemia patients. *Leukemia* 2013; **27**: 1520–1526.
- 199 7 Jaras M, Johnels P, Hansen N, Ågerstam H, Tsapogas P, Rissler M *et al.* Isolation and  
200 killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-  
201 1 receptor accessory protein. *PNAS* 2010; **107**: 16280–16285.
- 202 8 Herrmann H, Sadovnik I, Cerny-Reiterer S, Rulicke T, Stefanzl G, Willmann M *et al.*  
203 Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid  
204 leukemia. *Blood* 2014; **123**: 3951–3962.
- 205 9 Nievergall E, Ramshaw HS, Yong ASM, Biondo M, Busfield SJ, Vairo G *et al.*  
206 Monoclonal antibody targeting of IL-3 receptor with CSL362 effectively depletes  
207 CML progenitor and stem cells. *Blood* 2014; **123**: 1218–1228.
- 208 10 Florian S, Sonneck K, Hauswirth AW, Krauth M-T, Schernthaner G-H, Sperr WR *et al.*  
209 Detection of molecular targets on the surface of CD34+/CD38– stem cells in various  
210 myeloid malignancies. *Leuk Lymphoma* 2006; **47**: 207–222.
- 211 11 Levescot A, Flamant S, Basbous S, Jacomet F, Feraud O, Anne Bourgeois E *et al.* BCR-  
212 ABL-Induced Deregulation of the IL-33/ST2 Pathway in CD34(+) Progenitors from  
213 Chronic Myeloid Leukemia Patients. *Cancer Research* 2014; **74**: 2669–2676.
- 214 12 Hjorth-Hansen H, Stenke L, Söderlund S, Dreimane A, Ehrencrona H, Gedde-Dahl T  
215 *et al.* Dasatinib induces fast and deep responses in newly diagnosed chronic  
216 myeloid leukaemia patients in chronic phase: clinical results from a randomised  
217 phase-2 study (NordCML006). *European Journal of Haematology* 2014.  
218 doi:10.1111/ejh.12423.
- 219 13 Marin D, Ibrahim AR, Lucas C, Gerrard G, Wang L, Szydlo RM *et al.* Assessment of  
220 BCR-ABL1 Transcript Levels at 3 Months Is the Only Requirement for Predicting  
221 Outcome for Patients With Chronic Myeloid Leukemia Treated With Tyrosine

- 222 Kinase Inhibitors. *Journal of Clinical Oncology* 2012; **30**: 232–238.
- 223 14 Hanfstein B, Muller MC, Hehlmann R, Erben P, Lauseker M, Fabarius A *et al.* Early  
224 molecular and cytogenetic response is predictive for long-term progression-free  
225 and overall survival in chronic myeloid leukemia (CML). *Leukemia* 2012; **26**: 2096–  
226 2102.
- 227 15 Janssen JJWM, Deenik W, Smolders KGM, van Kuijk BJ, Pouwels W, Kelder A *et al.*  
228 Residual normal stem cells can be detected in newly diagnosed chronic myeloid  
229 leukemia patients by a new flow cytometric approach and predict for optimal  
230 response to imatinib. *Leukemia* 2011; **26**: 977–984.
- 231

232 **Figure legends**

233 **Figure 1**

234 Flow cytometry analysis of stem cell surface marker expression in CML. (a) Gating  
235 algorithm used to determine progenitor and stem cell compartments. (b)  
236 Representative histograms showing expression of IL1RAP, CD25, CD26, CD123,  
237 IL1R1, CD117 and ST2 in the CD34<sup>+</sup>CD38<sup>low</sup> compartment of a CML patient and a  
238 NBM. Isotype control (red line) and staining antibody (blue line). (c) Dotplots of co-  
239 expression of CD25 and CD26 in the CD34<sup>+</sup>CD38<sup>low</sup> compartment in 5 CML patients,  
240 isotype control (red) and staining antibody (blue).

241

242 **Figure 2**

243 Expression of IL1RAP, CD25 and CD123 in CD34<sup>+</sup>CD38<sup>low</sup> cells from 21 CML  
244 patients. (a) Histograms of IL1RAP, CD25 and CD123 in the CD34<sup>+</sup>CD38<sup>low</sup>  
245 compartment of a representative CML patient. Isotype control (red line) and staining  
246 antibody (blue line). (b) Co-expression of IL1RAP and CD25 in CD34<sup>+</sup>CD38<sup>low</sup> cells.  
247 (c) Flow cytometry assessed IL1RAP expression in CD34<sup>+</sup>CD38<sup>low</sup> cells shows a  
248 strong correlation to previously reported FISH data on *BCR/ABL1* expression from 21  
249 CML patients. (d) Flow cytometry assessed CD25 expression in CD34<sup>+</sup>CD38<sup>low</sup> cells  
250 shows a weaker correlation to the previously reported FISH data on *BCR/ABL1*. (e)  
251 CD34<sup>+</sup>CD38<sup>low</sup> cells from four CML patients sorted according to CD25 expression  
252 and analyzed with fluorescence in situ hybridization with probes detecting  
253 *BCR/ABL1*. Mean values are plotted, error bars show range.

Figure 1

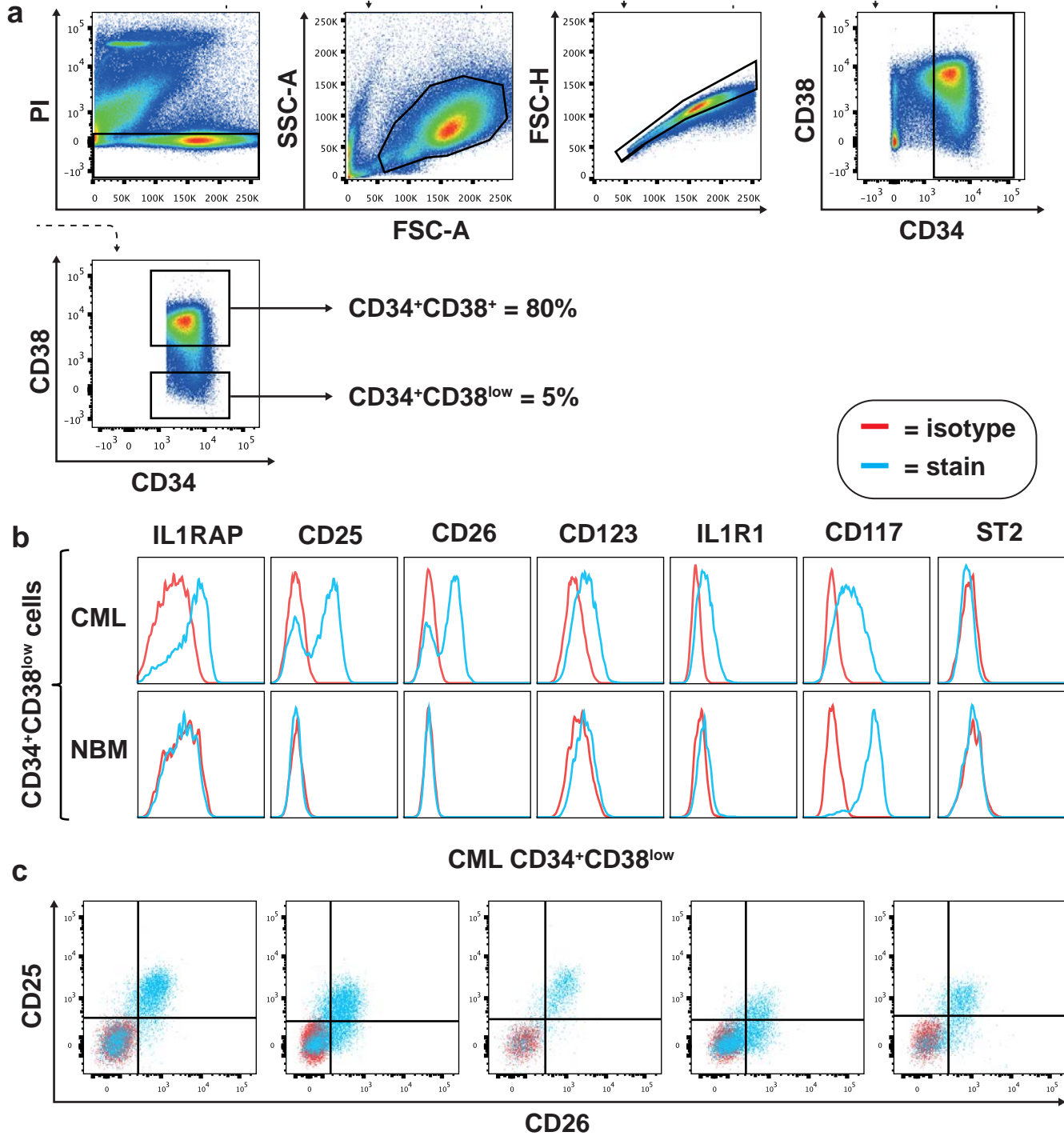
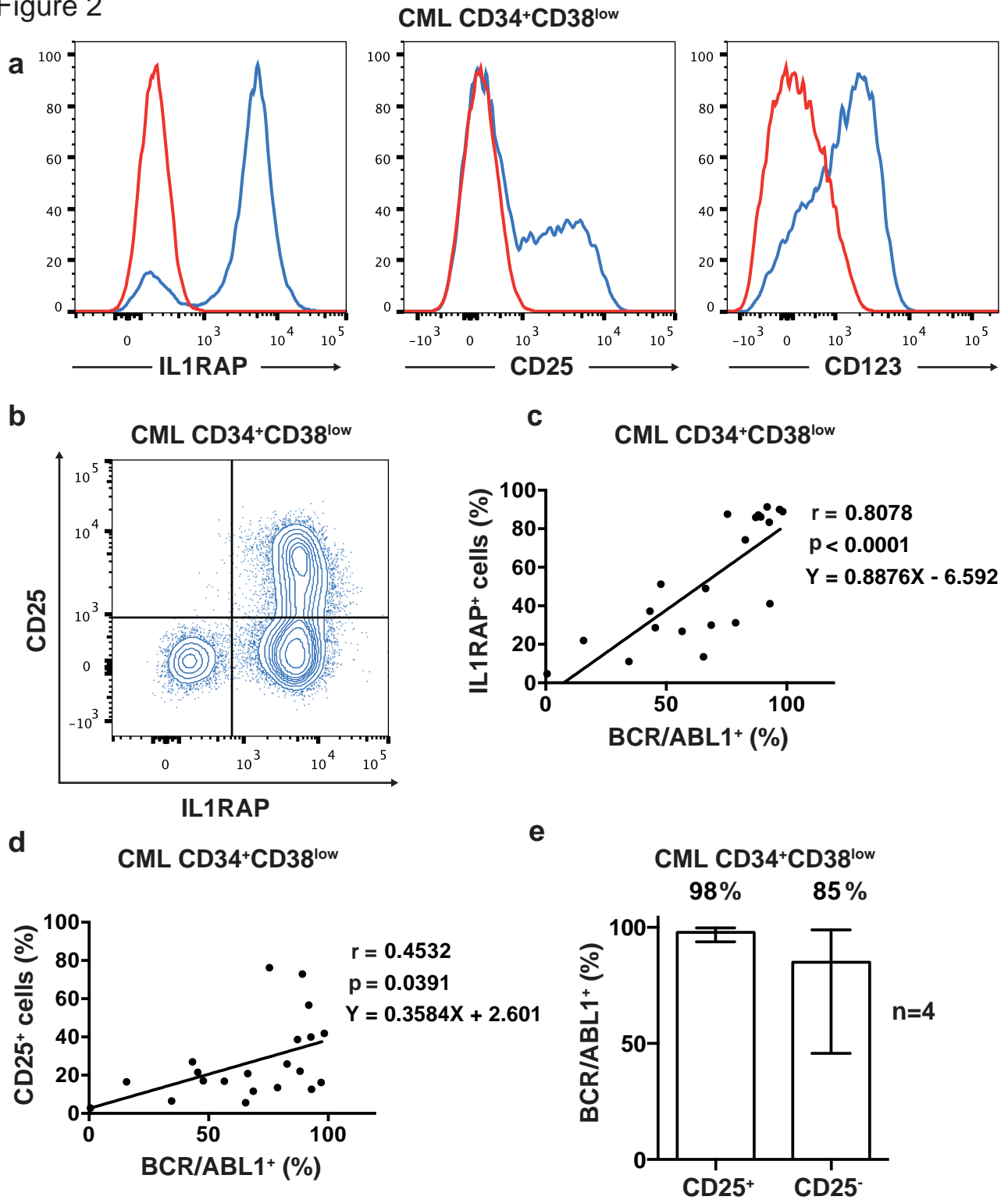


Figure 2



1 **Supplementary information to:**

2

3 **IL1RAP expression as a measure of leukemic stem cell burden at diagnosis of**  
4 **chronic myeloid leukemia predicts therapy outcome**

5

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## 19 **Supplementary materials and methods**

### 20 **Patient material**

21 All patient samples were obtained after informed, written consent and the study was  
22 performed in accordance to the declaration of Helsinki. Bone marrow aspirates from CP  
23 CML patients were obtained before initiation of TKI treatment, and mononuclear cells  
24 were isolated using Lymphoprep (GE Healthcare Bio-Sciences AB, Uppsala, Sweden).  
25 Enrichment for CD34 expressing cells was performed using MACS-beads according to  
26 the manufacturers instructions (Miltenyi Biotech, Bergisch Gladbach, Germany). Cells  
27 were cryopreserved until the time of analysis. Samples of peripheral blood and normal  
28 bone marrow (NBM) were processed in a similar way. In total, 26 diagnostic CML  
29 samples were included in the study. Twenty-one of these samples were from patients  
30 included in the NordCML006 study in which patients were randomized to receive  
31 treatment either with imatinib or dasatinib.<sup>1</sup> A summary of patient data included in the  
32 present study is provided in Supplementary Table 2.

33

### 34 **Flow cytometry analysis and cell sorting**

35 Analysis of cell surface protein expression was performed on a FACS Aria IIu (BD  
36 Biosciences, Franklin Lakes, New Jersey, USA). Antibodies used for staining (targeting  
37 CD34, CD38, IL1RAP, CD25, CD26, CD123, CD117, IL1R1 and ST2) were purchased from  
38 BD Biosciences, BioLegend (San Diego, California, USA) and R&D Systems (Minneapolis,  
39 Minnesota, USA); a full list of antibodies used is provided in Supplementary Table 4. One  
40 of the monoclonal anti-IL1RAP antibodies (used in combination with anti-CD25 and  
41 anti-CD123 antibodies) was purchased from R&D Systems (clone 89412) and  
42 conjugated to Biotin (BD Biosciences) by Innovagen AB (Lund, Sweden). Non-specific Fc-  
43 receptor binding was blocked by incubation with Fc-blocking IgG1 kappa from murine



44 myeloma, clone MOPC-21 (Sigma Aldrich, Saint Louis, Missouri, USA), and cells were  
45 stained in phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Waltham,  
46 Massachusetts, USA) with 2% fetal calf serum (FCS) (Stemcell Technologies, Cambridge,  
47 United Kingdom). Cell lines BV173, KU812 and KG1 (DSMZ, Braunschweig, Germany)  
48 were used as positive controls for CD34, CD38, IL1RAP, CD25, IL1R1, CD117 and ST2.  
49 Peripheral blood from a healthy donor was used as a positive control for CD26. Isotype  
50 controls were used at concentrations corresponding to the staining antibodies and  
51 fluorescence minus one (FMO) controls did not differ from isotype controls (data not  
52 shown). Propidium iodide (BD Biosciences) or Draq7 (Biostatus, Shephed, United  
53 Kingdom) were used as viability markers.

54           The 80% of cells with the highest CD38 expression were defined as positive  
55 and the bottom 5% with the lowest CD38 expression were defined as low, as previously  
56 described.<sup>2</sup> In one patient (patient 9) the lowest 2% were classified as CD38 low due to  
57 the atypical expression pattern of CD34 and CD38 (Supplementary Figure 3). Gates used  
58 to define positivity for a cell surface marker were set to include <1% false positive cells  
59 based on isotype controls. There were five exceptions due to poor separation and gates  
60 for these samples (patients 3, 7, 9, 18 and 19) were set manually (Supplementary Figure  
61 4). To compare flow cytometry data from this study to the data from FISH analysis  
62 previously performed in the NordCML006 study, two different gating algorithms to  
63 define CD34<sup>+</sup>CD38<sup>+</sup> and CD34<sup>+</sup>CD38<sup>low</sup> cells were used to match the two different gating  
64 strategies used for cell sorting at the different study centers (Figure 1a and  
65 Supplementary Figure 5).<sup>2</sup> A Flow-FISH sorting technique previously described was  
66 used to sort cells and analyze *BCR/ABL1* content by FISH.<sup>3</sup>

67

68 **Statistical analysis**

69 Statistical analyzes were performed using Graphpad Prism 6 (GraphPad Prism Inc, La  
70 Jolla, California, USA). Two-sided non-parametrical methods for correlations (Mann-  
71 Whitney U, Spearman's rank test) and tests of difference in distribution (Chi-square test)  
72 were used as deemed appropriate. In total, 26 patients were analyzed, 21 of whom had  
73 been included in the NordCML006 study and for whom detailed clinical data were  
74 available.<sup>1</sup> The remaining five patients were diagnosed at Skåne University Hospital and  
75 were not enrolled in the NordCML006 study.

76 **References**

- 77 1 Hjorth-Hansen H, Stenke L, Söderlund S, Dreimane A, Ehrencrona H, Gedde-Dahl T *et*  
78 *al.* Dasatinib induces fast and deep responses in newly diagnosed chronic myeloid  
79 leukaemia patients in chronic phase: clinical results from a randomised phase-2  
80 study (NordCML006). *European Journal of Haematology* 2014.  
81 doi:10.1111/ejh.12423.
- 82 2 Mustjoki S, Richter J, Barbany G, Ehrencrona H, Fioretos T, Gedde-Dahl T *et al.* Impact  
83 of malignant stem cell burden on therapy outcome in newly diagnosed chronic  
84 myeloid leukemia patients. *Leukemia* 2013; **27**: 1520–1526.
- 85 3 Jaras M, Johnels P, Hansen N, Ågerstam H, Tsapogas P, Rissler M *et al.* Isolation and  
86 killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1  
87 receptor accessory protein. *PNAS* 2010; **107**: 16280–16285.

88

89 **Supplementary tables**

90 **Supplementary Table 1.** Expression pattern of seven cell surface markers in the  
 91 CD34<sup>+</sup>CD38<sup>+</sup> and CD34<sup>+</sup>CD38<sup>low</sup> compartments of five chronic myeloid leukemia  
 92 patients and two normal bone marrow samples.

	CML CD34 <sup>+</sup> cells		NBM CD34 <sup>+</sup> cells	
	CD38 <sup>low</sup>	CD38 <sup>+</sup>	CD38 <sup>low</sup>	CD38 <sup>+</sup>
<b>IL1RAP</b>	100% (5/5)	100% (5/5)	0% (0/2)	100% (2/2)
<b>CD25</b>	100% (5/5)	0% (0/5)	0% (0/2)	0% (0/2)
<b>CD26</b>	100% (5/5)	0% (0/5)	0% (0/2)	0% (0/2)
<b>CD123</b>	80% (4/5)	60% (3/5)	50% (1/2)	100% (2/2)
<b>CD117</b>	100% (5/5)	100% (5/5)	100% (2/2)	100% (2/2)
<b>IL1R1</b>	100% (5/5)	100% (5/5)	50% (1/2)	100% (2/2)
<b>ST2</b>	20% (1/5)	20% (1/5)	0% (0/2)	0% (0/2)

93 Abbreviations: CML, chronic myeloid leukemia; NBM, normal bone marrow  
 94

95 **Supplementary Table 2.** Percentage of IL1RAP and CD25 positive cells in the  
 96 CD34<sup>+</sup>CD38<sup>low</sup> cell populations, percentage of *BCR/ABL1* positive cells determined  
 97 by fluorescence in situ hybridization in the CD34<sup>+</sup>CD38<sup>low</sup> cell populations, treatment  
 98 regimen, and the *BCR/ABL1* transcript levels at follow up in 21 newly diagnosed CML  
 99 patients.

Patient	CD34 <sup>+</sup> CD38 <sup>low</sup> cells			Drug	<i>BCR/ABL1</i> on IS (%)	
	IL1RAP <sup>+</sup> (%)	CD25 <sup>+</sup> (%)	<i>BCR/ABL1</i> <sup>+</sup> (%)		3 months	6 months
1	36.4	26.3	43	IM	0.7391	0.1011
2	24.9	13.6	79	IM	4.7036	2.0153
3	84.1	41.8	93	DAS	3.7763	1.3264
4	85.9	38.7	87	DAS	0.7807	0.0198
5	19.7	10.8	69	IM	0.4734	0.1957
6	74.3	25.9	83	DAS	0.0076	0.0000
7	93.2	22.8	97	IM	2.6932	2.9056
8	12.1	5.36	66	IM	0.0634	0.0184
9	57.8	22.4	66	DAS	0.0000	0.0077
10	4.78	2.81	1	DAS	0.0526	0.0166
11	22	16.5	16	IM	3.2648	0.3582
12	11.1	6.49	35	DAS	0.2248	0.0699
13	28.6	21.5	45	IM	1.2560	0.0406
14	34.6	13.7	93	DAS	0.0125	0.0000
15	50	15.7	48	IM	0.0730	0.0115
16	86.1	72.9	89	IM	4.3748	0.2785
17	28.5	20.5	57	DAS	0.1161	0.0776
18	91.2	42.6	98	IM	0.2326	0.0324
19	90.5	23.4	88	DAS	1.9723	0.0000
20	91.4	56.7	92	IM	1.3660	11.2385
21	87.6	76.2	75	IM	89.5865	22.8885

100 Abbreviations: IS, International Scale; IM, imatinib; DAS, dasatinib

101

102 **Supplementary Table 3.** Clinical response rates in CML patients stratified into  
 103 IL1RAP<sup>low</sup> and IL1RAP<sup>high</sup> based on expression as a measure of LSC-burden at  
 104 diagnosis.

	<i>BCR/ABL1</i> ≤10% at 3 months		CCyR at 3 months		<i>BCR/ABL1</i> ≤0.1% at 3 months		<i>BCR/ABL1</i> ≤1% at 6 months	
	IL1RAP expression status at diagnosis <sup>a</sup>							
	Low	High	Low	High	Low	High	Low	High
<b>Yes</b>	13	7	13	4	6	0	12	4
<b>No</b>	0	1	0	2	7	8	1	4
<b>P-value</b>	ns		<0.001		0.023		0.048	

105 <sup>a</sup>IL1RAP<sup>low</sup> was defined as <80% of CD34<sup>+</sup>CD38<sup>low</sup> cells expressing IL1RAP and

106 IL1RAP<sup>high</sup> as ≥80% of CD34<sup>+</sup>CD38<sup>low</sup> cells expressing IL1RAP.

107 Abbreviations: CCyR, complete cytogenetic remission; ns, not significant

108

109 **Supplementary Table 4.** Complete list of antibodies used for flow cytometry

110 analysis.

<b>Target</b>	<b>Fluorochrome</b>	<b>Clone</b>	<b>Company</b>
CD34	BV421	Mono-mouse IgG1 581	BD Biosciences
CD38	BV605	Mono-mouse IgG1 HB7	BD Biosciences
IL1RAP	APC	Mono-mouse IgG1 89412	R&D Systems
CD25	PE-CF594	Mono-mouse IgG1 M-A251	BD Biosciences
CD26	FITC	Mono-mouse IgG2a L272	BD Biosciences
CD123	BV711	Mono-mouse IgG1 9F5	BD Biosciences
CD117	Pe-Cy7	Mono-mouse IgG1 140D2	BioLegend
IL1R1	PE	Poly -goat IgG	R&D Systems
ST2	PE	Mono-mouse IgG1	R&D Systems
CD34	APC	Mono-mouse IgG1 581	BioLegend
CD38	APC-H7	Mono-mouse IgG1 HB7	BD Biosciences
CD25	PE	Mono-mouse IgG1 M-A251	BD Biosciences
IL1RAP	(Biotin)	Mono-mouse IgG1 89412	R&D Systems
Biotin	BV421	(Streptavidin)	BD Biosciences
Isotype	BV421	Mono-mouse IgG1 MOPC-21	BioLegend
Isotype	BV605	Mono-mouse IgG1 X40	BD Biosciences
Isotype	APC	Mono-mouse IgG1 MOPC-21	BioLegend
Isotype	PE-CF594	Mono-mouse IgG1 X40	BD Biosciences
Isotype	FITC	Mono-mouse IgG2a MOPC-173	BioLegend
Isotype	BV711	Mono-mouse IgG1 MOPC-21	BioLegend
Isotype	Pe-Cy7	Mono-mouse IgG1 MOPC-21	BioLegend
Isotype	PE	Poly -goat IgG	R&D Systems
Isotype	PE	Mono-mouse IgG1 97203	BioLegend
Isotype	APC	Mono-mouse IgG1 MOPC-21	BioLegend
Isotype	APC-H7	Mono-mouse IgG1 MOPC-21	BD Biosciences
Isotype	PE	Mono-mouse IgG1 97203	BioLegend
Isotype	BV711	Mono-mouse IgG1 MOPC-21	BioLegend
Isotype	(Biotin)	Mouse Isotyp - Biotin, Clone 11711	R&D Systems
Viability	Draq7	-	Biostatus
Viability	PI	-	BD Biosciences

111

112 **Supplementary figure legends**

113 **Supplementary Figure 1.**

114 Expression of IL1RAP, CD25 and CD123 in the CD34<sup>+</sup>CD38<sup>+</sup> and CD34<sup>+</sup>CD38<sup>low</sup>  
115 cells of two normal bone marrow samples. Isotype control (red line) and staining  
116 antibody (blue line).

117

118 **Supplementary Figure 2**

119 CD34<sup>+</sup>CD38<sup>low</sup> cells from a CML patients sorted according to IL1RAP expression and  
120 analyzed with fluorescence in situ hybridization with probes detecting *BCR/ABL1*.

121

122 **Supplementary Figure 3**

123 The left plot shows the gates used to determine CD34<sup>+</sup>CD38<sup>+</sup> progenitor population  
124 and CD34<sup>+</sup>CD38<sup>low</sup> stem cell containing population. The right plot shows gates used  
125 to determine CD34<sup>+</sup>CD38<sup>+</sup> progenitor population and CD34<sup>+</sup>CD38<sup>low</sup> stem cell  
126 containing population for patient no 9. The 2% cells with lowest CD38 expression  
127 were defined as CD38<sup>low</sup> in this patient.

128

129 **Supplementary Figure 4**

130 Gates for IL1RAP and CD25 expression were set to include less than 1% of the  
131 isotype control. For five samples this was not possible, instead gates to determine  
132 the size of the positive fraction were set manually as shown here. Cell populations  
133 depicted are CD34<sup>+</sup>CD38<sup>low</sup>, isotype control in red and stained samples in blue.

134



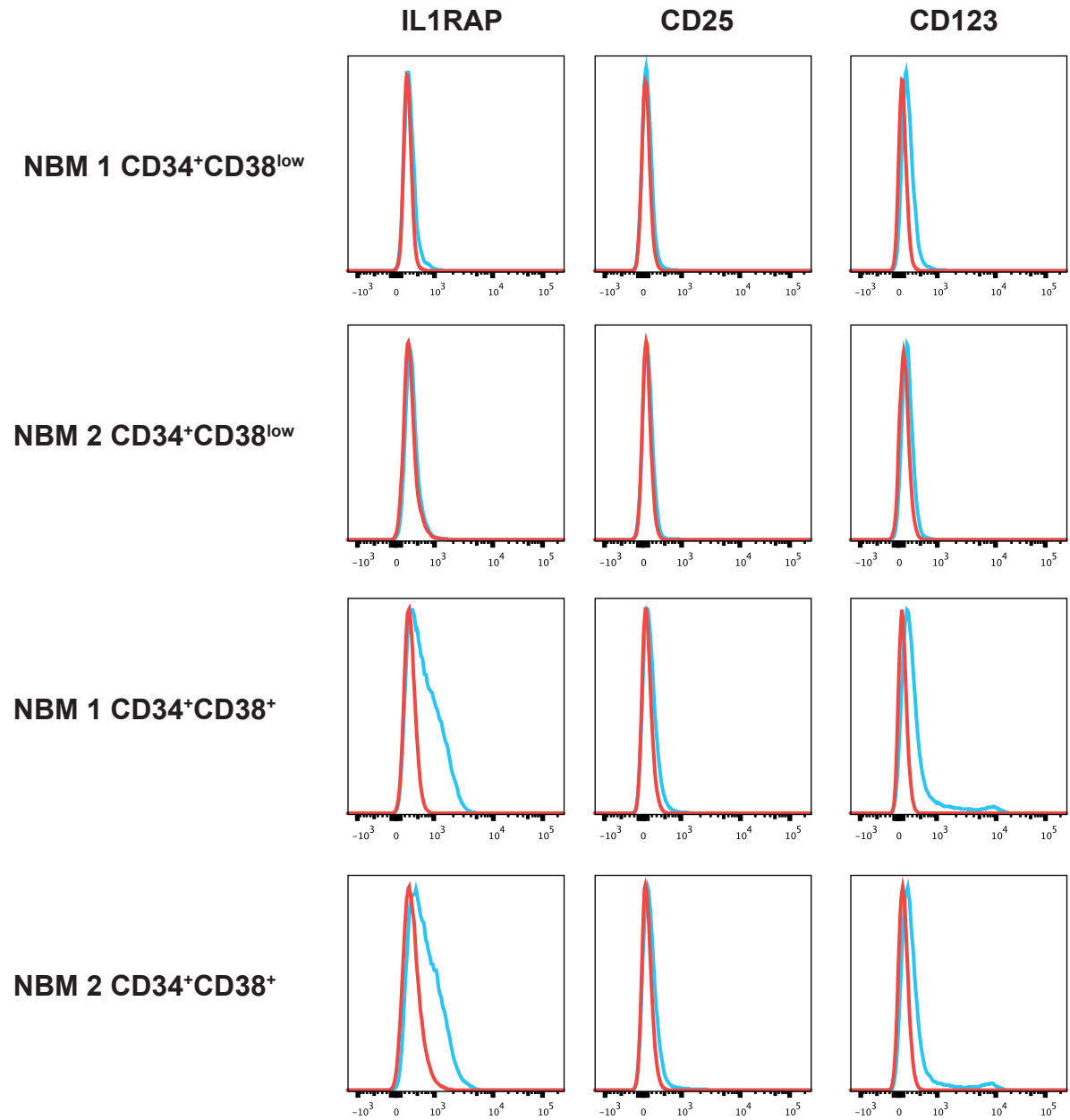
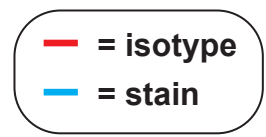
135 **Supplementary Figure 5**

136 Gating algorithm used for chronic myeloid leukemia samples collected in Finland,

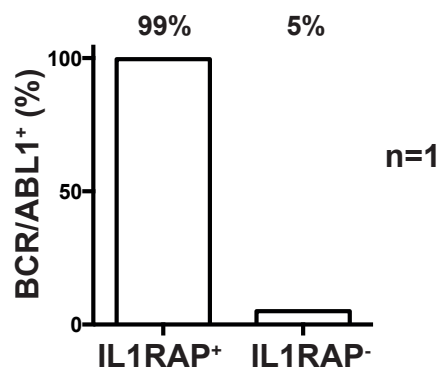
137 matching the gates used when sorting these cells for FISH.

138

# Supplementary Figure 1

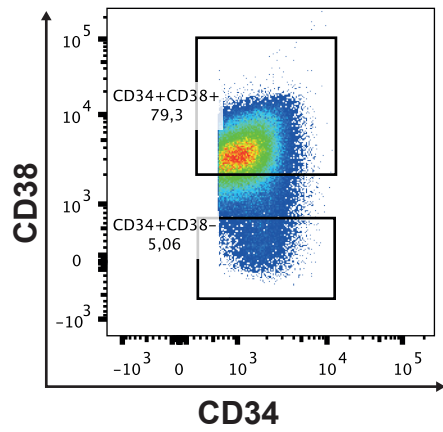


Supplementary Figure 2

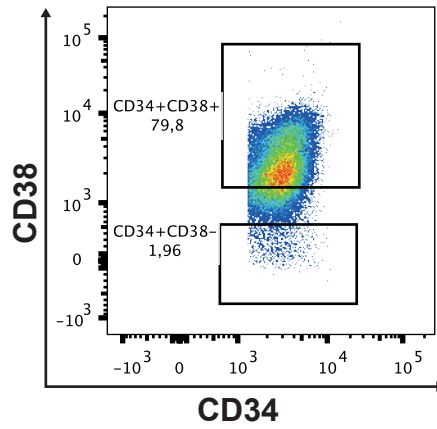


# Supplementary Figure 3

## Standard gates



## Patient 9



# Supplementary Figure 4

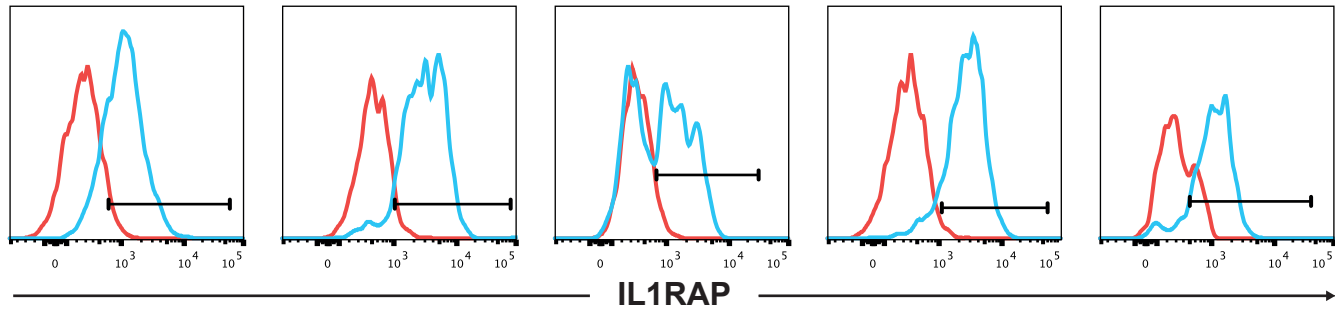
**Patient 3**

**Patient 7**

**Patient 9**

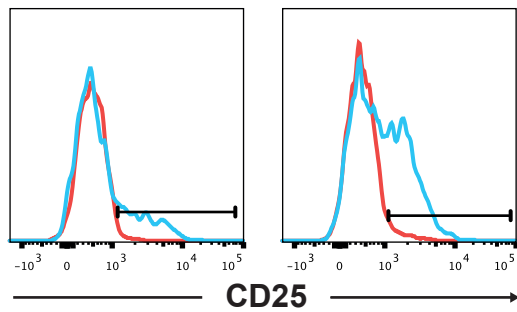
**Patient 18**

**Patient 19**



**Patient 7**

**Patient 18**



— = isotype

— = stain

# Supplementary Figure 5

