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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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Conflicts of interest

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

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LETTER TO THE EDITOR

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

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

Several markers have recently been proposed to be upregulated on candidate CML stem cells, including IL1RAP,[7] CD25 (IL2RA),[8] CD26 (DPP4),[8] CD123 (IL3RA),[9] , CD117(KIT),[10] and ST2 (IL1RL1 or IL33R).[11] Of these markers, only CD26 and IL1RAP, a co-receptor of the interleukin 1 receptor (IL1R1) with unknown function in normal and malignant hematopoiesis, have so far been proven to separate *BCR/ABL1* positive from negative cells within the CD34$^+$CD38^{low} fraction.[7,8] Here, we first used a staining protocol to simultaneously analyze the expression of these seven markers in bone marrow aspirates from five CML patients and two normal bone marrows (NBM). The LSC-containing CML CD34$^+$CD38^{low} compartment was defined as shown in Figure 1a. We found that IL1RAP, CD25 and CD26 were distinctly upregulated on primitive CML cells relative to corresponding cells in NBM, whereas the other markers showed a variable expression pattern or were expressed also on CD34$^+$CD38^{low} NBM cells (Figure 1b and Supplemental Table 1). CD123, CD117 and IL1R1 were all expressed on the immature CML CD34$^+$CD38^{low} cells but showed expression also on the normal HSC. In contrast to a recent study reporting low ST2 expression on CML CD34$^+$CD38^{low} cells,[11] this marker could only be detected in one of five CML patients using the same antibody, possibly because of a lower sensitivity of our flow cytometry protocol. Thus IL1RAP, CD25 and CD26 were specifically expressed on CML CD34$^+$CD38^{low} cells compared to corresponding normal cells.

To study the expression of LSC markers in more detail, IL1RAP, CD25 and CD123 were chosen for further analysis using an optimized panel of antibodies. As CD25 and CD26 displayed similar expression patterns in primitive CML cells (Figure 1c), consistent with previous studies,[8] we included only CD25 in the staining protocol. Instead, we selected CD123 since antibodies targeting this receptor currently are being evaluated as a therapy in CML.[9] These three markers were analyzed in a cohort of 21 CML patients included in the NordCML006 study in which patients were randomized to receive treatment either with imatinib or dasatinib.[12] A summary of patient data is provided in Supplementary Table 2. Using the optimized protocol, we obtained a clear separation of both IL1RAP positive and CD25 positive cells within the CML CD34$^+$CD38^{low} compartment, whereas CD123 expression failed to divide cells into distinct positive and negative cell populations (Figure 2a). In NBM, we did not observe expression of IL1RAP or CD25 in the $CD34^+CD38^{low}$ population, while CD123 was weakly expressed (Supplementary Figure 1). When analyzing co-expression of IL1RAP and CD25 in the CML $CD34^+CD38^{low}$ compartment, we found one population co-expressing IL1RAP and CD25 and one expressing only IL1RAP. No cells were found to express only CD25 (Figure 2b). Although the cell fractions expressing IL1RAP and CD25 showed a significant correlation (Spearman's Rho 0.8558, $p<0.0001$), IL1RAP was present on a consistently larger fraction of CML $CD34^+CD38^{low}$ cells (mean of difference 25.62%, CI 95% 15.81-35.44, $p<0.0001$).

To further delineate the difference between cells expressing IL1RAP and CD25, we correlated the flow cytometry data on IL1RAP and CD25 expression from the current study to previously reported *BCR/ABL1* FISH data on the CML $CD34^+CD38^{low}$ population from the same patients.[6] IL1RAP expression showed a strong correlation with *BCR/ABL1* positivity in the CML $CD34^+CD38^{low}$ fraction ($r=0.8078$, $p<0.0001$), with a slope of regression line close to 1 ($Y=0.8876X-6.6$)(Figure 2c). This indicates that almost all $CD34^+CD38^{low}$ *BCR/ABL1* positive cells express IL1RAP and that the *BCR/ABL1* negative cells lack IL1RAP. Only a weak correlation was seen between *BCR/ABL1* positive cells and CD25 expression ($r=0.4532$, $p=0.0391$) with a slope of regression of 0.36 ($Y=0.3584X+2.6$), indicating that $CD34^+CD38^{low}$ *BCR/ABL1* positive cells often lack CD25 expression (Figures 2d). We then used a previously described Flow-FISH sorting technique to sort $CD34^+CD38^{low}$ cells according to CD25 expression and evaluated the fraction *BCR/ABL1* positive cells in four CML patients. We found that of the CD25 expressing cells, a mean of 98% were *BCR/ABL1* positive cells (ranging between 94-100%), whereas the CD25 negative population of the same patients still contained a mean of 85% *BCR/ABL1* positive cells (range between 46-99%). Consistent with our previous findings,[7] sorted IL1RAP positive $CD34^+CD38^{low}$ cells contained a great majority (99%) of *BCR/ABL1* positive cells (Supplementary Figure 2), whereas IL1RAP negative cells were almost all *BCR/ABL1* negative (5%). Taken together, these data show that IL1RAP is a marker for *BCR/ABL1* positive cells in the CML $CD34^+CD38^{low}$ fraction, whereas CD25 is only expressed on a subfraction of *BCR/ABL1* positive cells.

The response of CML to TKI treatment is currently monitored by cytogenetic analyses and real time quantitative reverse polymerase chain reaction (RQ-PCR) analysis of *BCR/ABL1* transcript levels.[4] However, so far no easily implementable laboratory-based method is available to estimate the LSC-burden at diagnosis as a possible predictor of treatment response. We therefore investigated whether IL1RAP or CD25 expression in the CML $CD34^+CD38^{low}$ compartment, as a measure of the LSC-burden at diagnosis, could provide clinically relevant prognostic information. In our previous study, the presence of ≥80% *BCR/ABL1* positive cells by FISH in the $CD34^+CD38^{low}$ fraction at diagnosis of CML was used to define a group of patients with 'high' LSC-burden, whereas <80% were designated as 'low' LSC-burden.[6] Based on IL1RAP expression, we used a similar definition, i.e. patients were classified as $IL1RAP^{low}$ (n=13, defined as <80% of the $CD34^+CD38^{low}$ cells expressing IL1RAP) or $IL1RAP^{high}$ (n=8, ≥80% of $CD34^+CD38^{low}$ cells expressing IL1RAP). Following this subdivision, we investigated if the two groups differed in the probability of achieving optimal response as defined by the European Leukemia Net (ELN) guidelines, following TKI treatment (Supplementary Table 3).[4] All $IL1RAP^{low}$ and 7 of 8 $IL1RAP^{high}$ patients achieved *BCR/ABL1* transcript levels ≤10% within 3 months. However, $IL1RAP^{low}$ patients had a higher chance of reaching transcript levels <1% after 6 months ($p=0.0475$), a definition of optimal response according to ELN.[4] Since this cohort consisted of mainly optimal responders at 3 months, we looked at even deeper levels of molecular and cytogenetic response, previously shown to predict long-term outcome at this time point.[13,14] Interestingly, IL1RAP^{low} patients (13 out of a total of 21) had a higher chance of achieving complete cytogenetic response (CCyR) ($p=0.0009$) and major molecular response (MMR; *BCR/ABL1* transcript levels ≤0.1%) after 3 months ($p=0.023$) (Supplementary Table 3). No significant correlations were seen between IL1RAP expression and Sokal or Hasford risk scores. Altogether, this suggests that IL1RAP expression as a measure of LSC-burden at diagnosis predicts cytogenetic and molecular response to TKI treatment although larger prospective studies are needed before such measurement can be implemented clinically.

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

In conclusion, we identified IL1RAP, CD25 and CD26 as the most specific markers for primitive CML cells relative to corresponding normal cells and demonstrate that the percentage of IL1RAP expressing cells within the CD34^{+}CD38^{low} compartment can be used to predict the response to TKI treatment. The described flow cytometry-based protocol may become a valuable prognostic tool in the management of CML patients.

Supplementary information is available at Leukemia’s website

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Figure legends

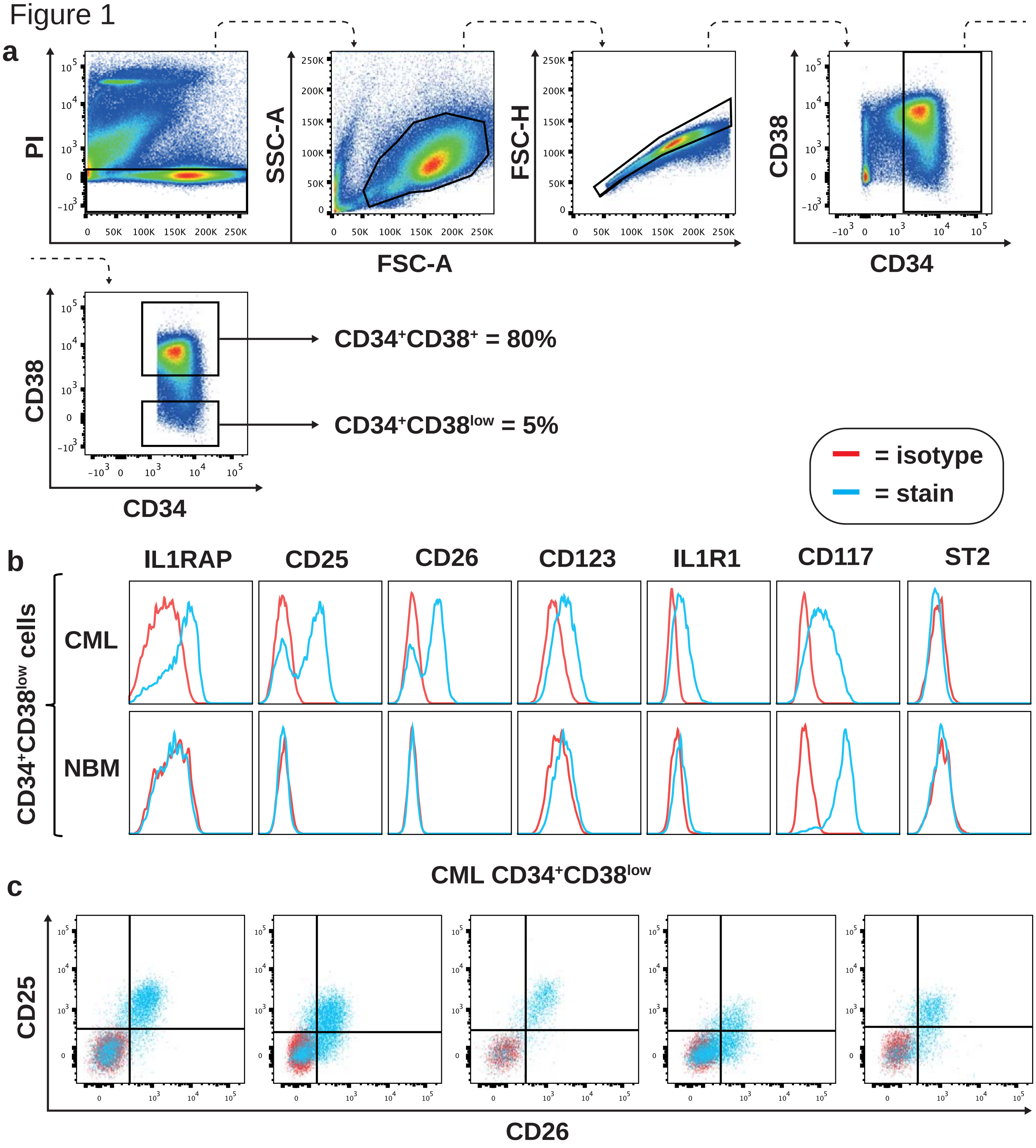
Figure 1

Flow cytometry analysis of stem cell surface marker expression in CML. (**a**) Gating algorithm used to determine progenitor and stem cell compartments. (**b**) Representative histograms showing expression of IL1RAP, CD25, CD26, CD123, IL1R1, CD117 and ST2 in the $CD34^{+}CD38^{low}$ compartment of a CML patient and a NBM. Isotype control (red line) and staining antibody (blue line). (**c**) Dotplots of co-expression of CD25 and CD26 in the $CD34^{+}CD38^{low}$ compartment in 5 CML patients, isotype control (red) and staining antibody (blue).

Figure 2

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

Figure 1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PI
SSC-A
FSC-H
CD38
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CD34
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50K
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250K
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10^4
10^3
-10^3
CD34⁺CD38⁺ = 80%
CD34⁺CD38^low = 5%
= isotype
= stain
b
IL1RAP
CD25
CD26
CD123
IL1R1
CD117
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CD34⁺CD38^low cells
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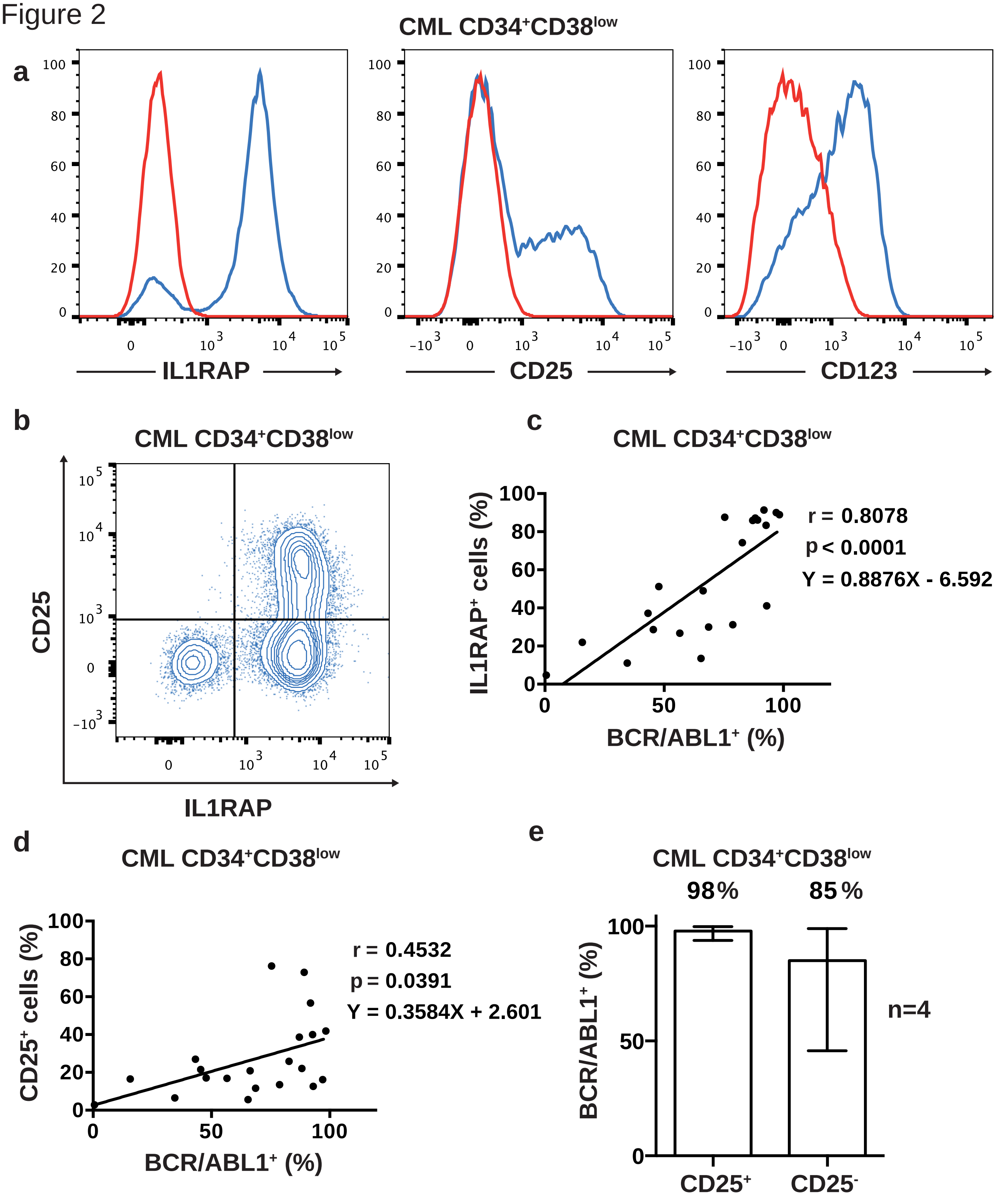
CML CD34⁺CD38^low
CD25
CD26

Figure 2



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CML CD34+CD38low
100
80
60
40
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0
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10 3
10 4
10 5
IL1RAP
-10 3
CD25
CD123
b
CML CD34+CD38low
CD25
IL1RAP
c
CML CD34+CD38low
IL1RAP+ cells (%)
BCR/ABL1+ (%)
r = 0.8078
p < 0.0001
Y = 0.8876X - 6.592
d
CML CD34+CD38low
CD25+ cells (%)
BCR/ABL1+ (%)
r = 0.4532
p = 0.0391
Y = 0.3584X + 2.601
e
CML CD34+CD38low
98 %
85 %
BCR/ABL1+ (%)
n=4
CD25+
CD25-

Supplementary information to:

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

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Supplementary materials and methods

Patient material

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

Flow cytometry analysis and cell sorting

Analysis of cell surface protein expression was performed on a FACS Aria IIu (BD Biosciences, Franklin Lakes, New Jersey, USA). Antibodies used for staining (targeting CD34, CD38, IL1RAP, CD25, CD26, CD123, CD117, IL1R1 and ST2) were purchased from BD Biosciences, BioLegend (San Diego, California, USA) and R&D Systems (Minneapolis, Minnesota, USA); a full list of antibodies used is provided in Supplementary Table 4. One of the monoclonal anti-IL1RAP antibodies (used in combination with anti-CD25 and anti-CD123 antibodies) was purchased from R&D Systems (clone 89412) and conjugated to Biotin (BD Biosciences) by Innovagen AB (Lund, Sweden). Non-specific Fc-receptor binding was blocked by incubation with Fc-blocking IgG1 kappa from murine myeloma, clone MOPC-21 (Sigma Aldrich, Saint Louis, Missouri, USA), and cells were stained in phosphate-buffered saline (PBS) (Thermo Fisher Scientific, Waltham, Massachusetts, USA) with 2% fetal calf serum (FCS) (Stemcell Technologies, Cambridge, United Kingdom). Cell lines BV173, KU812 and KG1 (DSMZ, Braunschweig, Germany) were used as positive controls for CD34, CD38, IL1RAP, CD25, IL1R1, CD117 and ST2. Peripheral blood from a healthy donor was used as a positive control for CD26. Isotype controls were used at concentrations corresponding to the staining antibodies and fluorescence minus one (FMO) controls did not differ from isotype controls (data not shown). Propidium iodide (BD Biosciences) or Draq7 (Biostatus, Shepshed, United Kingdom) were used as viability markers.

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

Statistical analysis

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

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Supplementary tables

Supplementary Table 1. Expression pattern of seven cell surface markers in the CD34$^+$CD38$^+$ and CD34$^+$CD38^{low} compartments of five chronic myeloid leukemia patients and two normal bone marrow samples.

	CML CD34$^+$ cells		**NBM CD34$^+$ cells**	
	CD38^{low}	**CD38$^+$**	**CD38^{low}**	**CD38$^+$**
IL1RAP	100% (5/5)	100% (5/5)	0% (0/2)	100% (2/2)
CD25	100% (5/5)	0% (0/5)	0% (0/2)	0% (0/2)
CD26	100% (5/5)	0% (0/5)	0% (0/2)	0% (0/2)
CD123	80% (4/5)	60% (3/5)	50% (1/2)	100% (2/2)
CD117	100% (5/5)	100% (5/5)	100% (2/2)	100% (2/2)
IL1R1	100% (5/5)	100% (5/5)	50% (1/2)	100% (2/2)
ST2	20% (1/5)	20% (1/5)	0% (0/2)	0% (0/2)

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

Supplementary Table 2. Percentage of IL1RAP and CD25 positive cells in the CD34$^+$CD38^{low} cell populations, percentage of *BCR/ABL1* positive cells determined by fluorescence in situ hybridization in the CD34$^+$CD38^{low} cell populations, treatment regimen, and the *BCR/ABL1* transcript levels at follow up in 21 newly diagnosed CML patients.

	CD34$^+$CD38^{low} cells				***BCR/ABL1* on IS (%)**	
Patient	**IL1RAP$^+$(%)**	**CD25$^+$(%)**	**BCR/ABL1$^+$(%)**	**Drug**	**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

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

Supplementary Table 3. Clinical response rates in CML patients stratified into IL1RAP$^{\text{low}}$ and IL1RAP$^{\text{high}}$ based on expression as a measure of LSC-burden at diagnosis.

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

[a] IL1RAP$^{\text{low}}$ was defined as <80% of CD34^{+}CD38$^{\text{low}}$ cells expressing IL1RAP and IL1RAP$^{\text{high}}$ as ≥80% of CD34^{+}CD38$^{\text{low}}$ cells expressing IL1RAP.

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

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

Target	Fluorochrome	Clone	Company
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

Supplementary figure legends

Supplementary Figure 1.

Expression of IL1RAP, CD25 and CD123 in the $CD34^{+}CD38^{+}$ and $CD34^{+}CD38^{low}$ cells of two normal bone marrow samples. Isotype control (red line) and staining antibody (blue line).

Supplementary Figure 2

$CD34^{+}CD38^{low}$ cells from a CML patients sorted according to IL1RAP expression and analyzed with fluorescence in situ hybridization with probes detecting *BCR/ABL1.*

Supplementary Figure 3

The left plot shows the gates used to determine $CD34^{+}CD38^{+}$ progenitor population and $CD34^{+}CD38^{low}$ stem cell containing population. The right plot shows gates used to determine $CD34^{+}CD38^{+}$ progenitor population and $CD34^{+}CD38^{low}$ stem cell containing population for patient no 9. The 2% cells with lowest CD38 expression were defined as $CD38^{low}$ in this patient.

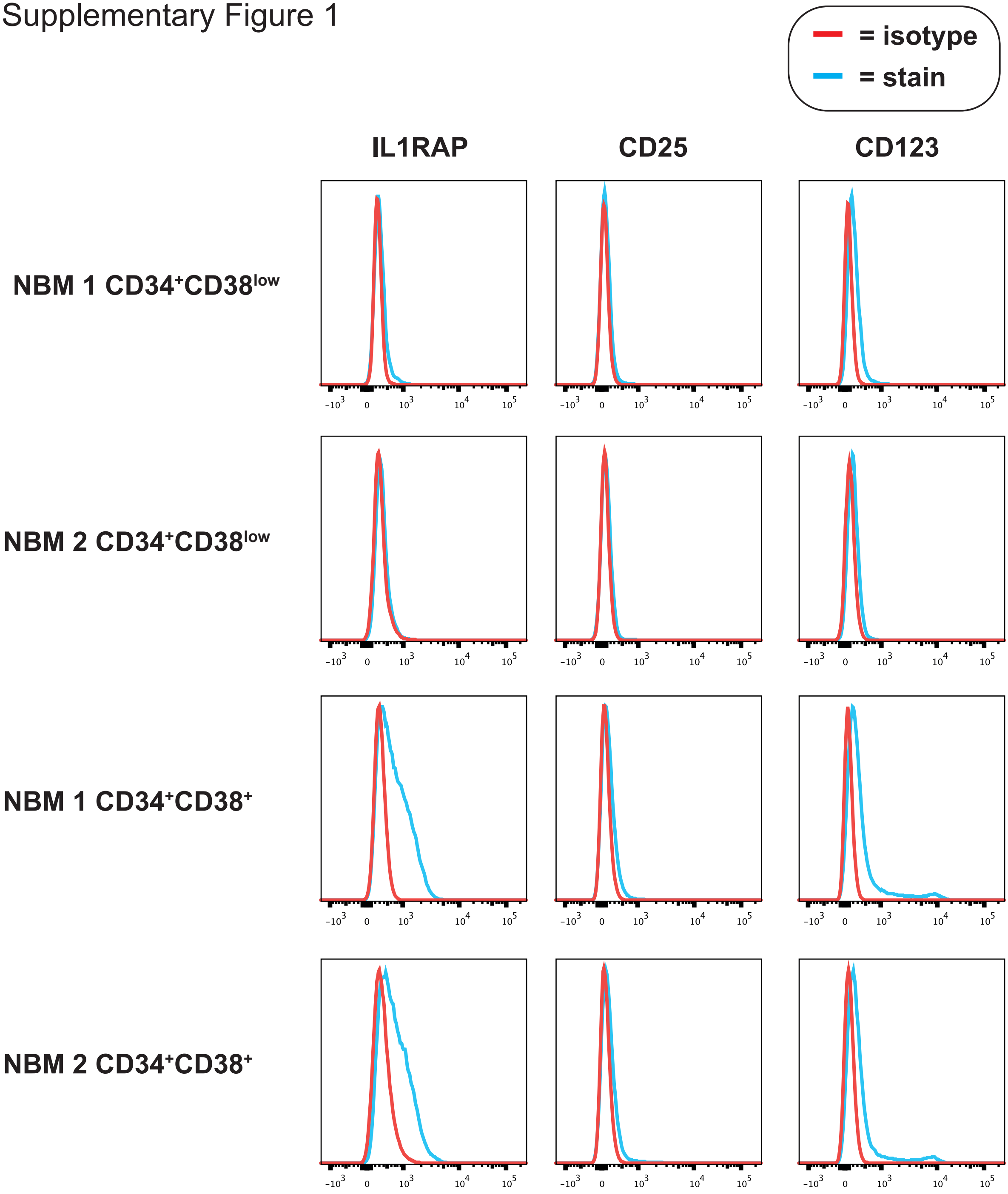
Supplementary Figure 4

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

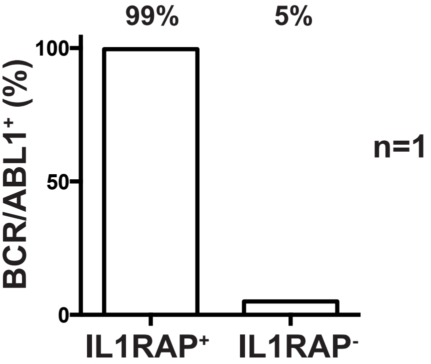
Supplementary Figure 5

Gating algorithm used for chronic myeloid leukemia samples collected in Finland, matching the gates used when sorting these cells for FISH.

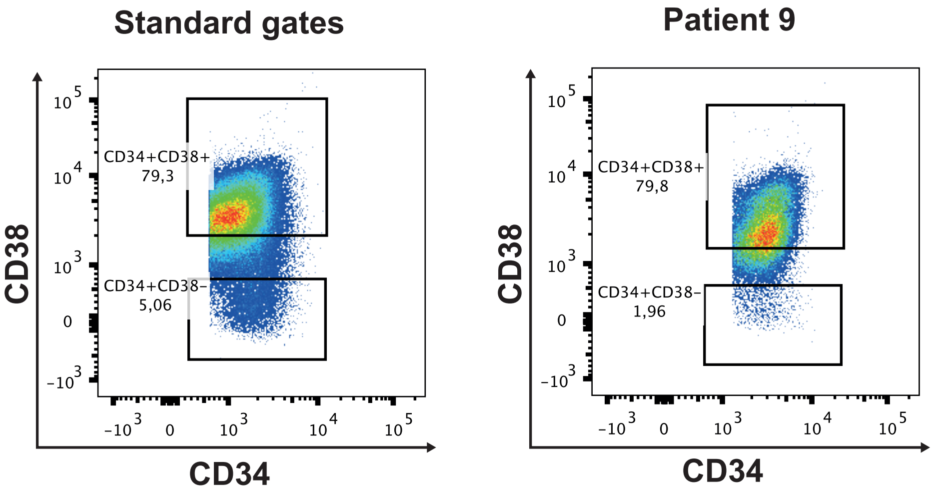
Supplementary Figure 1



Supplementary Figure 2

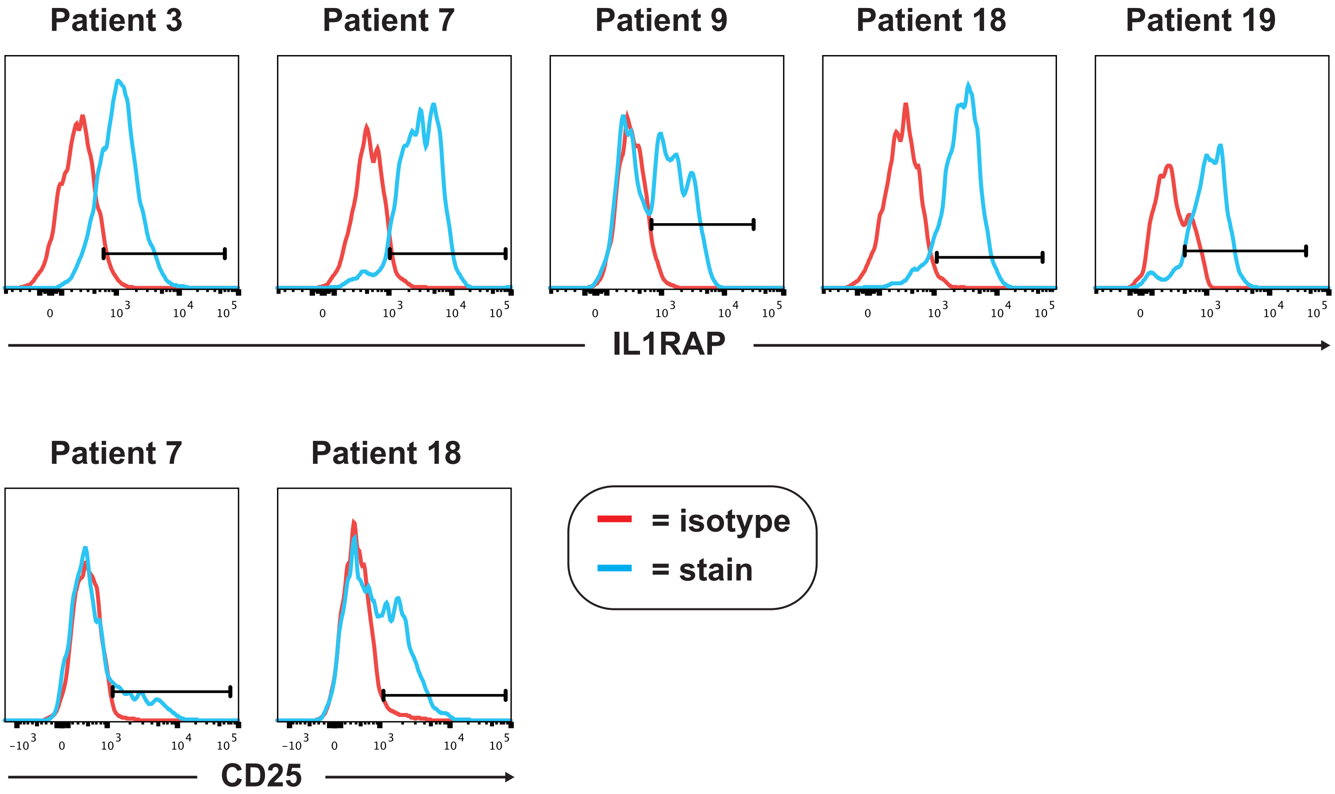


Supplementary Figure 3

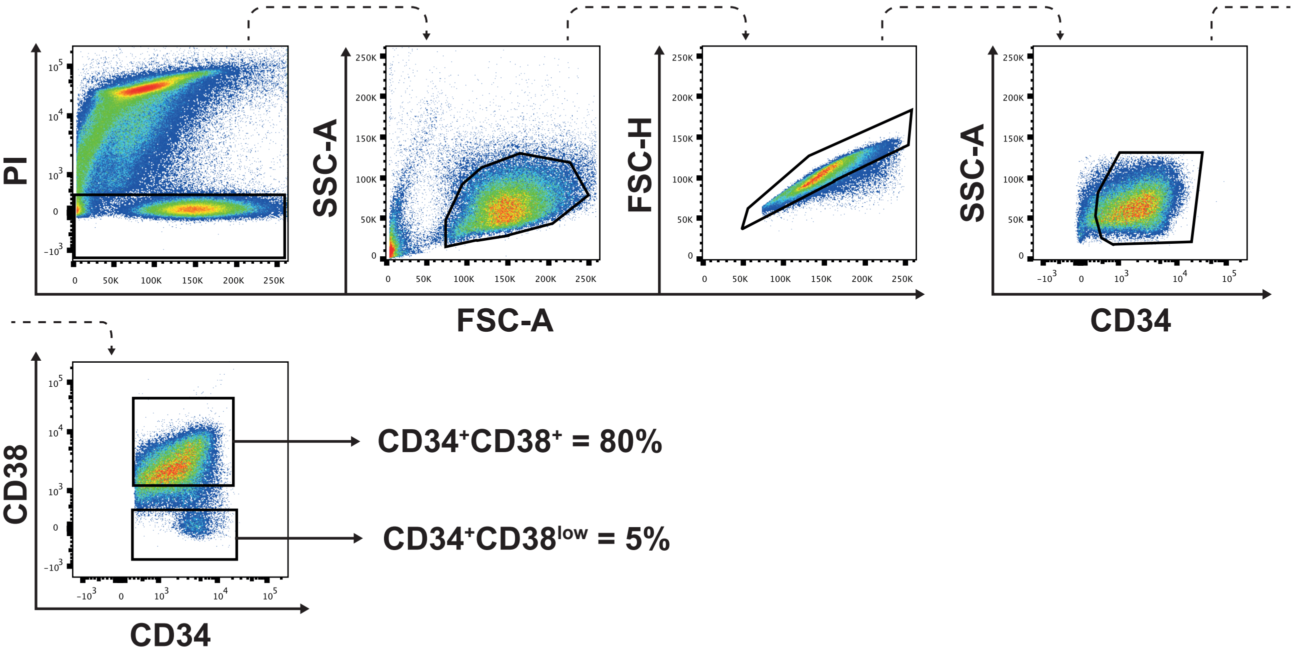

Standard gates

Patient 9

Supplementary Figure 4



Supplementary Figure 5



CD34$^+$CD38$^+$ = 80%

CD34$^+$CD38^{low} = 5%