Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein

Marcus Järås², Petra Johnels³, Nils Hansen², Helena Ågerstam³, Panagiotis Tsapogas⁵, Marianne Rissler³, Carin Lassen⁵, Tor Olofsson⁵, Ole Weis Bjerrum⁶, Johan Richter⁶, and Thoas Fioretos⁶,¹

¹Department of Clinical Genetics, University and Regional Laboratories, Skåne University Hospital, Lund University, 22185 Lund, Sweden; Departments of ²Hematology and ³Molecular Medicine and Gene Therapy, Lund University, 22184 Lund, Sweden; and ⁴Department of Hematology, Rigshospitalet, University of Copenhagen, 2100 Copenhagen, Denmark

Chronic myeloid leukemia (CML) was the first human neoplasm to be associated with a recurrent genetic aberration, the Philadelphia (Ph) chromosome, formed through a reciprocal translocation between chromosomes 9 and 22 and giving rise to the constitutively active tyrosine kinase P210 BCR/ABL1. Therapeutic strategies aiming for a cure of CML will require full eradication of Ph chromosome-positive (Ph⁺) CML stem cells. Here we used gene-expression profiling to identify IL-1 receptor accessory protein (IL1RAP) as up-regulated in CML CD34⁺ cells and also in cord blood CD34⁺ cells as a consequence of retroviral BCR/ABL1 expression. To test whether IL1RAP expression distinguishes normal (Ph⁻) and leukemic (Ph⁺) cells within the CML CD34⁺/CD38⁻ cell compartment, we established a unique protocol for conducting FISH on small numbers of sorted cells. By using this method, we sorted cells directly into drops on slides to investigate their Ph-chromosome status. Interestingly, we found that the CML CD34⁺/CD38⁻/IL1RAP⁺ cells were Ph⁻, whereas CML CD34⁺/CD38⁻/IL1RAP⁻ cells were almost exclusively Ph⁺. By performing long-term culture-initiating cell assays on the two cell populations, we found that Ph⁻ and Ph⁺ candidate CML stem cells could be prospectively separated. In addition, by generating an anti-IL1RAP antibody, we provide proof of concept that IL1RAP can be used as a target on CML CD34⁺/CD38⁻ cells to induce antibody-dependent cell-mediated cytotoxicity. This study thus identifies IL1RAP as a unique cell surface biomarker distinguishing Ph⁻ from Ph⁺ candidate CML stem cells and opens up a previously unexplored avenue for therapy of CML.

antibody-dependent cell-mediated cytotoxicity | cancer | biomarker | therapeutic antibody

Chronic myeloid leukemia (CML) was genetically characterized by the Philadelphia (Ph) chromosome, formed through a reciprocal translocation between chromosomes 9 and 22 and giving rise to the constitutively active tyrosine kinase P210 BCR/ABL1. Therapeutic strategies aiming for a cure of CML will require full eradication of Ph chromosome-positive (Ph⁺) CML stem cells. Here we used gene-expression profiling to identify IL-1 receptor accessory protein (IL1RAP) as up-regulated in CML CD34⁺ cells and also in cord blood CD34⁺ cells as a consequence of retroviral BCR/ABL1 expression. To test whether IL1RAP expression distinguishes normal (Ph⁻) and leukemic (Ph⁺) cells within the CML CD34⁺/CD38⁻ cell compartment, we established a unique protocol for conducting FISH on small numbers of sorted cells. By using this method, we sorted cells directly into drops on slides to investigate their Ph-chromosome status. Interestingly, we found that the CML CD34⁺/CD38⁻/IL1RAP⁺ cells were Ph⁻, whereas CML CD34⁺/CD38⁻/IL1RAP⁻ cells were almost exclusively Ph⁺. By performing long-term culture-initiating cell assays on the two cell populations, we found that Ph⁻ and Ph⁺ candidate CML stem cells could be prospectively separated. In addition, by generating an anti-IL1RAP antibody, we provide proof of concept that IL1RAP can be used as a target on CML CD34⁺/CD38⁻ cells to induce antibody-dependent cell-mediated cytotoxicity. This study thus identifies IL1RAP as a unique cell surface biomarker distinguishing Ph⁻ from Ph⁺ candidate CML stem cells and opens up a previously unexplored avenue for therapy of CML.

Global Gene Expression Analysis Identifies IL1RAP as Up-Regulated in CML CD34⁺ Cells. Much effort has been put into investigations aimed at identifying a cell-surface biomarker for Ph⁺ CML stem cells, as reviewed by Jiang et al. (15). However, so far, no cell-surface marker has been identified that would allow prospective separation of CML stem cells from normal HSCs. To search for up-regulated genes encoding cell-surface proteins on primitive CML cells, we performed global transcriptional profiling of CD34⁺ cells from 10 chronic-phase CML patients and six healthy donors. Genes identified as up-regulated in CML were matched to the Gene Ontology (GO) category “integral to plasma membrane” (see Materials and Methods for details). In total, 13 up-regulated genes in CML CD34⁺ cells matched to the selected GO category (Fig. 1A). To identify up-regulated genes more directly linked to P210 BCR/ABL1 expression, we performed gene-expression analysis of cord blood CD34⁺ cells following retroviral P210 BCR/ABL1 expression in parallel. This analysis resulted in 23 up-regulated genes matching to the same GO category list (Fig. 1B). Interestingly, one gene, IL1RAP, was strongly up-regulated in both CML and in CD34⁺ cells as a consequence of P210 BCR/ABL1 expression. The occurrence of eradicating them. Encouraging reports in this direction have suggested that this strategy may be more efficient at identifying a cell-surface biomarker for Ph⁺ candidate CML stem cells. With the development of an assay for detecting BCR/ABL1 in low numbers of sorted cells and through long-term culturing-initiating cell (LTC-IC) assays, we further show that IL1RAP is a cell surface biomarker for putative CML stem cells. This finding is unique in allowing the prospective separation of such cells from normal HSCs. Finally, we generated an IL1RAP-targeting antibody that killed CML CD34⁺/CD38⁻ cells, but not corresponding normal cells, through antibody-dependent cell-mediated cytotoxicity (ADCC), demonstrating a unique concept for the possible eradication of CML stem cells.

Results

Global Gene Expression Analysis Identifies IL1RAP as Up-Regulated in CML CD34⁺ Cells. Much effort has been put into investigations aimed at identifying a cell-surface biomarker for Ph⁺ CML stem cells, as reviewed by Jiang et al. (15). However, so far, no cell-surface marker has been identified that would allow prospective separation of CML stem cells from normal HSCs. To search for up-regulated genes encoding cell-surface proteins on primitive CML cells, we performed global transcriptional profiling of CD34⁺ cells from 10 chronic-phase CML patients and six healthy donors. Genes identified as up-regulated in CML were matched to the Gene Ontology (GO) category “integral to plasma membrane” (see Materials and Methods for details). In total, 13 up-regulated genes in CML CD34⁺ cells matched to the selected GO category (Fig. 1A). To identify up-regulated genes more directly linked to P210 BCR/ABL1 expression, we performed gene-expression analysis of cord blood CD34⁺ cells following retroviral P210 BCR/ABL1 expression in parallel. This analysis resulted in 23 up-regulated genes matching to the same GO category list (Fig. 1B). Interestingly, one gene, IL1RAP, was strongly up-regulated in both CML and in CD34⁺ cells as a consequence of P210 BCR/ABL1 expression. The occurrence of

Author contributions: M.J. and T.F. provided the concept and design; M.J., P.J., N.H., H.Å., P.T., M.R., C.L., T.O., and J.R. performed research; O.W.B. and J.R. contributed new reagents/analytic tools; M.J., J.R., and T.F. analyzed data; M.J. and T.F. wrote the paper.

Conflict of interest statement: M.J. and T.F are the inventors of patent applications to cover IL1RAP for diagnostic and therapeutic applications in leukemia. M.J. and T.F. have equity ownership in a company (Cantargia AB, Ideon Research Park, Lund, Sweden) formed together with Lund University Bioscience AB. Cantargia AB is the owner of the patent applications mentioned above.

This article is a PNAS Direct Submission. Freely available online through the PNAS open access option.

¹To whom correspondence should be addressed. E-mail: thoas.fioretos@med.lu.se.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1004408107/-/DCSupplemental.
Flow-Drop-FISH Shows That IL1RAP Expression Separates Normal and Leukemic Cells Within CML CD34<sup>+</sup>-CD38<sup>−</sup> Cells. To test whether IL1RAP expression distinguishes Ph<sup>−</sup> and Ph<sup>+</sup> cells within the CD34<sup>+</sup>-CD38<sup>−</sup> cell compartment in CML, we applied FISH to detect the BCR/ABL1 rearrangement in cells sorted according to their IL1RAP expression (Fig. 3B). Because all Ph<sup>+</sup> cells are BCR/ABL1<sup>+</sup> whereas normal CML cells are BCR/ABL1<sup>−</sup>, we established a unique protocol for sorting CML cells based on their IL1RAP expression (Fig. 3B). This protocol was validated by using Flow-drop-FISH, which allowed the sorting of small numbers of CD34<sup>+</sup>-CD38<sup>−</sup> cells using standard protocols (20). Hence, to test whether IL1RAP expression distinguishes normal (Ph<sup>−</sup>) and leukemic (Ph<sup>+</sup>) cells within the CD34<sup>+</sup>-CD38<sup>−</sup> cell compartment, we applied this protocol for conducting FISH on small numbers of sorted cells (Materials and Methods). The first step in this protocol is based partly on a method for sorting cells into drops on slides followed by single-cell immunostaining (21). With this unique protocol involving cell sorting directly into drops on slides followed by FISH, we could sort as few as 30 cells into a drop, from which 15 nuclei were successfully scored by FISH (Fig. 4). Interestingly, by using Flow-drop-FISH, we found that the LTC-CFCs derived from CD34<sup>+</sup>-CD38<sup>−</sup> LTC-IC cells were BCR/ABL1<sup>+</sup> (99.7 ± 0.2% Ph<sup>+</sup>, n = 5), whereas CD34<sup>+</sup>-CD38<sup>−</sup> IL1RAP<sup>−</sup> cells were almost exclusively BCR/ABL1<sup>−</sup> (97.1 ± 3.4% Ph<sup>−</sup>, n = 5) (Fig. 4). These data show that IL1RAP expression separates leukemic and normal cells within the CD34<sup>+</sup>-CD38<sup>−</sup> cell compartment of CML patients at diagnosis.

IL1RAP Expression Distinguishes Ph<sup>+</sup> from Ph<sup>−</sup> Candidate CML Stem Cells. Chronic-phase CML stem cells have been characterized as a unique leukemia-associated antigen for being a unique leukemia-associated antigen on primitive CML cells. The finding of increased IL1RAP expression is in accordance with previous findings reporting transcriptional profiling of primitive CML cells (16, 17). The up-regulation of the IL1RAP transcript in CML CD34<sup>+</sup> cells was confirmed by real-time PCR (Materials and Methods). The first step in this protocol is based partly on a method for sorting cells into drops on slides followed by single-cell immunostaining (21). With this unique protocol involving cell sorting directly into drops on slides followed by FISH, we could sort as few as 30 cells into a drop, from which 15 nuclei were successfully scored by FISH (Fig. 4). Interestingly, by using Flow-drop-FISH, we found that the LTC-CFCs derived from CD34<sup>+</sup>-CD38<sup>−</sup> LTC-IC cells were BCR/ABL1<sup>+</sup> (99.7 ± 0.2% Ph<sup>+</sup>, n = 5), whereas CD34<sup>+</sup>-CD38<sup>−</sup> IL1RAP<sup>−</sup> cells were almost exclusively BCR/ABL1<sup>−</sup> (97.1 ± 3.4% Ph<sup>−</sup>, n = 5) (Fig. 4). These data show that IL1RAP expression separates leukemic and normal cells within the CD34<sup>+</sup>-CD38<sup>−</sup> cell compartment of CML patients at diagnosis.

IL1RAP Is Induced as a Consequence of Retroviral P210 BCR/ABL1 Expression and Is Also Present on a Population of CD34<sup>+</sup>-CD38<sup>−</sup> Cells from CML Patients. IL-1-induced IL-1 receptor-type 1 (IL-1R1) activation has previously been shown to stimulate colony growth of IFN-sensitive CML cells (16, 18). IL1RAP expression is in accordance with previous findings reporting transcriptional profiling of primitive CML cells (16, 17). The up-regulation of the IL1RAP transcript in CML CD34<sup>+</sup> cells was confirmed by real-time PCR (Fig. 1C). IL1RAP expression is presented as fold change in relation to NBM-C.

IL1RAP is important in regulating IL1RAP expression, either directly or through an indirect effect. Next, we investigated the cell-surface IL1RAP expression on CML CD34<sup>+</sup>-CD38<sup>−</sup> progenitor cells from five CML patients. In this subpopulation of cells, up-regulation of IL1RAP was observed compared with low IL1RAP expression in corresponding normal bone-marrow cells (Fig. 3A and Fig. S1). We then turned to the more immature CD34<sup>+</sup>-CD38<sup>−</sup> cell compartment of normal cells containing the HSCs. In agreement with the results of a previous study of normal primitive hematopoietic cells, this population displayed low or absent IL1RAP expression (Fig. 3D) (19). Strikingly, the CD34<sup>+</sup>-CD38<sup>−</sup> cells from CML patients, harboring both Ph<sup>−</sup> and Ph<sup>+</sup> CML stem cells, could be divided into two populations: one exhibiting low or absent IL1RAP expression and the other having higher IL1RAP expression (Fig. 3B). The IL1RAP<sup>+</sup> cell fraction constituted between 75% and 95% of the CD34<sup>+</sup>-CD38<sup>−</sup> cells (n = 5), corresponding to about 1 in 1,300 mononuclear cells; the more rare CD34<sup>+</sup>-CD38<sup>−</sup> IL1RAP<sup>+</sup> cells corresponded to about 1 in 11,000 mononuclear cells.
these data show that IL1RAP is a unique cell-surface biomarker that can be used to separate Ph" from Ph" CML LTC-IC.

**Antibody Targeting of IL1RAP on CML CD34+CD38− Cells Directs Natural Killer Cells to ADCC.** Several therapeutic antibodies, such as Rituximab directed against CD20, are believed to at least partially exert their therapeutic effect through ADCC (24). To test whether ADCC could be achieved using IL1RAP as a target, we generated a polyclonal rabbit anti-human IL1RAP antibody, designated KMT-1; we selected rabbit antibodies because human immune-system cells efficiently recognize the Fc domains of such antibodies, in contrast to goat and mouse antibodies (25). The ability of KMT-1 and a control IgG antibody to induce cell death was investigated using human natural killer (NK) cells as effector cells.

In the IL1RAP"Ph" leukemia cell line KG-1, lacking IL1RAP expression (Fig. 6B and Fig. S2A and B), only a low level of ADCC was observed, even at high KMT-1 concentrations (Fig. 6B). In contrast, in the Ph" CML cell line KU812, expressing IL1RAP (Fig. 6A and Fig. S2A and B), relatively high ADCC was observed in the presence of KMT-1 (Fig. 6B), demonstrating that KMT-1 has the potential to induce ADCC by binding IL1RAP on leukemic target cells. Moreover, blocking IL1RAP using the goat anti-IL1RAP antibody before the addition of KMT-1' caused a significant reduction in the ADCC effect, supporting the theory that KMT-1 induces ADCC in an IL1RAP-dependent manner (Fig. 6B). In addition, we assessed the IL1RAP expression in six more CML cell lines that all stained positively for IL1RAP (Fig. S4). On primary cells from CML patients and normal controls, KMT-1 showed a slightly weaker but similar staining pattern as that obtained with the previously used polyclonal goat anti-human IL1RAP antibody (Fig. S5). Immature cells from CML-1, CML-3, and CML-4 were tested in ADCC assays in parallel to cells from healthy control samples. In CML CD34+ cells, the binding of KMT-1 mediated ADCC at higher levels than in normal CD34+ control cells, correlating with the expression level of IL1RAP, in particular at lower antibody concentrations (Fig. 6C). More strikingly, among the stem cell-enriched CD34+CD38− cells, KMT-1 did not induce ADCC of normal CD34+CD38− cells, whereas a clear dose-dependent ADCC effect was observed in CML CD34+CD38− cells (Fig. 6D).

To address the selectivity of IL1RAP-targeting antibodies, we also characterized the IL1RAP expression on various cell populations of normal hematopoiesis. With the exception of monocytes showing a substantial IL1RAP expression, IL1RAP expression was relatively low or absent on most types of normal progenitor and mature cell populations (Fig. S6).

**Discussion**

In the present study, we have identified IL1RAP as a unique cell surface biomarker that distinguishes candidate CML stem cells from normal HSCs and used this knowledge to induce an antibody-dependent cell killing of CML CD34+CD38− cells. This finding opens up a previously unexplored possible strategy to treat CML by direct targeting of the CML stem cells, a concept that is distinct from the TKI therapies currently used, which preferentially are effective against cells downstream of the CML stem cells (8, 26).

The reason that CML stem cells are partially resistant to TKIs, such as imatinib (Gleevec, Novartis Inc.) is unclear, but factors that have been suggested to contribute to this resistance include quiescence, relatively high levels of BCR/ABL1 expression, ac-

---

**Fig. 2.** The kinase activity of P210 BCR/ABL1 induces up-regulation of IL1RAP on the cell surface. Flow cytometric analysis confirmed that IL1RAP expression is induced upon retroviral P210 BCR/ABL1 expression of cord blood CD34+ cells, 3 d after transduction (A). CD34+GFP− cells were gated according to the gates in the contour plots. The histogram shows the expression of IL1RAP for negative-control staining (white), MIG control (green), MIG-P210 (blue), and MIG-P210 kinase inactive (KI) (light red). KU812 cells were also treated with 5 μM imatinib and IL1RAP expression was assessed 2 d later in live cells (7-AAD−) (B). The histogram shows the expression of IL1RAP for imatinib-treated cells (yellow) and nonimatinib-treated cells (gray). One representative experiment out of three is shown. MIG; MSCV-ires-GFP, murine stem cell-virus internal ribosomal entry site-green fluorescent protein.

**Fig. 3.** IL1RAP is up-regulated on the cell surface of CML CD34+CD38− cells. FACS analysis of CD34+ cells from five CML patients in chronic-phase (CML-1-5) and from two NBM samples (NBM1, -2). (A) FACS dot-plot showing gating for CD34+CD38− or CD34+CD38+ cells in a representative CML patient. (B) Histogram showing IL1RAP expression within CD34+CD38− cells. Light red represents control-stained samples and blue represents IL1RAP-stained samples. The sorting gates for CD34+CD38− "IL1RAP+" and CD34+CD38− "IL1RAP−" cells are outlined in the histograms. IL1RAP is up-regulated in CML CD34+CD38− cells compared with their normal counterparts.
required mutations in BCR/ABL1, and combinatorial expression of specific membrane transporter proteins in these cells (7, 8, 27–29). Given these features, novel treatment approaches to ultimately eradicate the CML stem cells are highly desirable. One such strategy is an antibody-based therapy directly targeting CML stem cells, in which the antibody mode of action is independent of the known resistance mechanisms causing CML stem cells to be unresponsive to TKI treatment. The major limitations for such developments have so far been the lack of a cell surface receptor distinguishing Ph+ from Ph− CML stem cells. By using global gene expression analyses of primary CML cells, we identified IL1RAP as a candidate target. IL1RAP is a member of the Toll-like receptor superfamily and is a well-known coreceptor of IL-1R1 (30). IL1RAP is thus crucial in mediating the effect of the proinflammatory cytokine IL-1, but it is also involved in mediating the signal of IL-33, a cytokine that activates T cells and mast cells through binding of its receptor ST2, which subsequently dimerizes with IL1RAP (31).

Although we did not address whether IL1RAP is functionally involved in leukemogenesis, we did find that IL1RAP becomes up-regulated on the cell surface of CB CD34+ cells following retroviral expression of BCR/ABL1. This result suggests that IL1RAP is regulated by the well-known signaling cascades that BCR/ABL1 activates (4, 32).

By using Flow-drop-FISH in sorted LTC-IC assays, we demonstrated that IL1RAP can be used for prospective separation of Ph+ and Ph− candidate CML stem cells. Although it would be desirable to test the stem-cell properties of these two cell populations in immunodecient mice, the low numbers of sorted CML cells acquired from the IL1RAP− and IL1RAP+ CD34+ CD38− cell subpopulations, together with the general low efﬁciency in engrafting chronic phase CML cells in these mice (33), prevented us from successfully performing such experiments. Nevertheless, the near-complete separation of Ph+ and Ph− CML cells based on IL1RAP expression within the CD34+ CD38− population and their separation in LTC-IC assays suggest that IL1RAP expression can be used to prospectively isolate Ph+ from Ph− candidate CML stem cells. It cannot entirely be excluded that extremely rare Ph+ CML stem cells might be contained within the CD34+ CD38− IL1RAP− cell fraction; however, because on average 97.1 ± 3.4% of the CD34+CD38+ IL1RAP− cells were Ph−, we ﬁnd this possibility less likely.

As demonstrated in this study, the ability to separate Ph+ from Ph− candidate CML stem cells should also enable future detailed mechanistic studies of these two cell populations in CML. In addition, the Flow-drop-FISH protocol could serve as a powerful method for characterizing genetic aberrations in small numbers of sorted cells, such as leukemic stem cells (34). Furthermore, in particular for CML in which leukemic cells can be traced by detection of BCR/ABL1 using FISH, Flow-drop-FISH can be used to monitor residual immature leukemic cells during treatment. Importantly, by generating an antibody that targets IL1RAP, we are unique in providing proof of concept that CML CD34+ CD38− cells can be targeted while preserving corresponding normal cells. Notably, we have not directly demonstrated that the ADCC effect exclusively targets the Ph+ CML cells within the CD34+ CD38− cell subset. However, given that on average 86% of the cells within the CD34+ CD38− subpopulation in the patients tested were Ph+, and the almost undetectable ADCC in CD34+ CD38− cells from normal controls, our data strongly support the idea that the IL1RAP-targeting antibodies indeed induce ADCC preferentially in Ph+ CML CD34+ CD38− cells.

Therapeutic antibodies are commonly administered intravenously, and selectivity and speciﬁcity becomes a major concern for reducing toxicity (24). IL1RAP expression was present in monocytes, but was low or absent in most types of normal bone-marrow progenitor and mature cell populations (35). Therefore, these cells should be monitored carefully, potential future therapeutic IL1RAP-targeting antibodies are expected to show low toxicity on normal hematopoietic cells. However, given the weak but present
IL1RAP expression in mature lymphocytes and the role of IL1RAP in IL-33–mediated activation of T lymphocytes and mast cells, these cell types should also be monitored carefully.

Finally, it is worth noting that because the antibody mode of action in ADCC is to direct immunological cells to target cell killing, the therapeutic mechanism here is independent of known mechanisms of TKI resistance in CML. Hence, we hypothesize that the concept of antibody-mediated killing of IL1RAP-expressing CML stem cells may also have the potential to eradicate such cells in patients, either alone or in combination with current regimens, ultimately leading to a cure for CML patients.

Materials and Methods

Collection of CML Patient Cells. Blood samples from CML patients at diagnosis were obtained after informed consent and before treatment was initiated, according to a protocol approved by the regional, research ethics committee, Lund University. Samples were received both from the Department of Hematology at Skåne University Hospital, Sweden, and from Rigshospitalet, Copenhagen, Denmark. For CML patient characteristics, see Table S1. Mononuclear cells were separated using Lymphoprep (Axis-Shield PoC AS) according to the manufacturer’s instructions, and CD34+ cells were enriched using the CD34+ cell isolation kit (Miltenyi Biotec), as previously described (35). This separation yielded a purity of CD34+ cells above 95%. CD34+ cells were split into two fractions; one fraction was washed in PBS, resuspended in TRIzol, and frozen at −80 °C, whereas the other fraction was frozen in liquid nitrogen. As reference samples, bone marrow samples from healthy volunteers were obtained after informed consent at the Skåne University Hospital, followed by CD34 cell enrichment as described above.

P210 BCR/ABL1 Expression in Cord Blood CD34+ Cells. Umbilical cord blood CD34+ cells were enriched and transduced as previously described (36). See SI Materials and Methods for details.

Microarray Analysis and Real-Time PCR. Microarray analyses on patient samples were performed using oligonucleotide arrays from the Swegiane DNA Microarray Resource Center at Lund University, Sweden. For details on the microarray analysis, see SI Materials and Methods. Real-time PCR analysis was performed using an ABI Prism 7500 analyzer (Applied Biosystems) and standard protocols. All samples were analyzed in triplicate. Primers and probes for IL1RAP were ordered from Applied Biosystems as assay-on-demand primers. The relative quantity was calculated based on the ΔΔCt method (37) and normalized to 18S rRNA.

Flow Cytometric Analysis. Flow cytometric analyses were performed in a FACS Canto flow cytometer, and flow cytometric cell sorting was done in a FACS Aria cell sorter (both from Becton-Dickinson Immunocytometry Systems). See SI Materials and Methods for details on the flow cytometric analysis.
Flow-Drop-FISH. Glass slides were treated with 0.01% poly-

1. Nowel P, Hungerford D (1960) A minute chromosome in human chronic granulocytic leukemia. Science 132:149.

2. Rayport JD (1980) Letter: A new consistent chromosomal abnormality in chronic myelogenous leukemia identified by quinacrine fluorescence and Giemsa staining. Nature 243:290–293.

3. Heisterkamp N, Stam K, Groffen J, de Klein A, Grosveld G (1985) Structural organization of the bcr gene and its role in the Ph translocation. Nature 315:758–761.

4. Deming NW, Goldman JM, Metc JV (2000) The molecular biology of chronic myeloid leukemia. Blood 96:3343–3356.

5. Fialkov PJ, Denman AM, Jacobson RJ, Lowenthal MN (1978) Chronic myelocytic leukemia. Origin of some lymphocytes from leukemia stem cells. J Clin Invest 62:815–823.

6. Kavalerich E, Goff D, Jamieson CH (2008) Chronic myeloid leukemia stem cells. J Clin Oncol 26:2911–2915.

7. Jin X, et al. (2007) Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leukemia 21:526–935.

8. Copland M, et al. (2006) Dasatinib (BMS-354825) targets an earlier progenitor cells of patients with CML in chronic phase. Blood 1091:2817–2824.

9. Jin L, Hope KJ, Zhai Q, Smadhja Joelle F, Dick JE (2006) Targeting of CD44 eradicates human acute myeloid leukemia stem cells. Nat Med 12:1167–1174.

10. Tavor S, et al. (2004) CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. Cancer Res 64: 2817–2824.

11. Jin L, et al. (2009) Monoclonal antibody-mediated targeting of CD123, IL-3 receptor alpha chain, eliminates human acute myeloid leukemia stem cells. Cell Stem Cell 5:31–42.

12. Majeti R, et al. (2009) CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. Cell 138:286–299.

13. Eisterer W, et al. (2000) Different subsets of primary chronic myeloid leukemia stem cells engraft immunodeficient mice and produce a model of the human disease. Leukemia 19:435–441.

14. Schmittgen TD, Livak KJ (2001) Analysis of relative gene expression data using real-time quantitative PCR. Nat Protoc 5:31–40.

15. Stricklin B, et al. (2008) Stem cell biomarkers in chronic myeloid leukemia. Dis Markers 24:201–216.

16. Díaz-Bánuelos B, et al. (2009) The hematopoietic stem cell in chronic phase CML is characterized by a transcriptional profile resembling normal myeloid progenitor cells and reflecting loss of quiescence. Leukemia 23:892–899.

17. Diáz-Blanco E, et al. (2007) Molecular signature of CD34+ hematopoietic stem and progenitor cells of patients with CML in chronic phase. Leukemia 21:494–504.

18. Estrov Z, et al. (1991) Suppression of chronic myelogenous leukemia colony growth by interleukin-1 (IL-1) receptor antagonist and soluble IL-1 receptors: A novel application for inhibitors of IL-1 activity. Blood 78:1476–1484.

19. Hystad ME, et al. (2007) Characterization of early stages of human B cell development by gene expression profiling. J Immunol 179:3662–3671.

20. Castor A, et al. (2005) Distinct patterns of hematopoietic stem cell involvement in acute lymphoblastic leukemia. Nat Med 11:630–637.

21. Ema H, et al. (2009) Antigenic hematopoietic stem cells: Purification and single-cell assays. Nat Protoc 1:2979–2987.

22. Jiang X, Lopez A, Holyoake T, Eaves A, Eaves C (1999) Autorocrine production and action of IL-3 and granulocyte-colony-stimulating factor in chronic myeloid leukemia. Proc Natl Acad Sci USA 96:8835–8839.

23. Petzer AL, et al. (1996) Characterization of primitive subpopulations of normal and leukemic cells present in the blood of patients with newly diagnosed as well as established chronic myeloid leukemia. Blood 88:2162–2171.

24. Morris JC, Waldmann TA (2009) Antibody-based therapy of leukemia. Expert Rev Mol Med 11:e29.

25. Peters CJ, Theofilopoulos AN (1977) Antibody-dependent cellular cytotoxicity against murine leukemia viral antigens: Studies with human lymphoblastoid cell lines and human peripheral lymphocytes as effector cells comparing rabbit, goat, and mouse antiserum. J Immunol Methods 30:317–327.

26. Jorgensen HG, Allan EK, Jordanides NE, Mountford JC, Holyoake TL (2007) Nilotinib exerts equipotent antiproliferative effects to imatinib and does not induce apoptosis in CD34+ CML cells. Blood 109:4016–4019.

27. Gehrke M, et al. (2002) Chronic granulocytic leukemia, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood 99: 319–325.

28. Jiang X, et al. (2010) Properties of CD34+ CML stem/progenitor cells that correlate with different clinical responses to imatinib mesylate. Blood doi 10.1182/blood-2009-05-222471.

29. Engler JR, et al. (2010) Chronic myeloid leukemia CD34+ cells have reduced uptake of imatinib due to low OCT-1 activity. Leukemia 24:765–770.

30. Subramaniam S, Stanberg C, Cunningham C (2004) The interleukin 1 receptor family. Dev Comp Immunol 28:415–428.

31. Ali S, et al. (2007) IL-1 receptor accessory protein is essential for IL-33-induced activation of 1 lymphocytes and mast cells. Proc Natl Acad Sci USA 104:18660–18665.

32. Ren R (2005) Mechanisms of BCR-ABL in the pathogenesis of chronic myelogenous leukemia. Nat Rev Cancer 5:172–183.

33. Chalandon Y, et al. (2005) BCR-ABL-transduced human cord blood cells produce abnormal populations in immunodeficient mice. Leukemia 19:442–448.

34. Dick JE (2008) Stem cell concepts renew cancer research. Blood 112:4793–4807.

35. Nilsson M, Karlsson S, Fan X (2004) Functionally distinct subpopulations of cord blood CD34+ cells are transduced by adenoviral vectors with serotype 5 or 35 tropism. Mol Ther 5:377–385.

36. Järás A, et al. (2009) Expression of P190 and P210 BCR-ABL1 in normal human CD34+ cells induces similar gene expression profiles and results in a STAT5-dependent expansion of the erythroid pathway. Exp Hematol 37:367–375.

37. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta CT) method. Methods 25:402–408.

38. Barbouti A, et al. (2003) Clinical and genetic studies of ETV6/ABL1-positive chronic myeloid leukaemia. Br J Haematol 120:85–93.

39. Hoge DE, Lansdorp PM, Reid D, Gerhard B, Eaves CJ (1996) Enhanced detection, quantification, and differentiation of primitive human hematopoietic cells in cultures containing murine fibroblasts engineered to produce human steel factor, interleukin-3, and granulocyte colony-stimulating factor. Blood 88:3765–3773.

40. Wilkinson RW, Lee-MacAry AE, Davies D, Snary D, Ross EL (2001) Antibody-dependent cell-mediated cytotoxicity. A flow cytometry-based assay using fluorophores. J Immunol Methods 258:183–191.

ACKNOWLEDGMENTS. We thank Ingridt Åström-Grundström, Hong Qian, and Marja Eklöf (Hematopoietic Stem Cell Laboratory, Lund University, Sweden) for technical guidance with the long-term culture-initiating cell assay; Connie Eaves and Terry Fox Laboratory (Vancouver, Canada) for providing the MIG-P210 BCR/ABL1 construct; Ravi Bhatia, Division of Stem Cell and Leukemia Research (City of Hope, Duarte, CA) for providing the MIG-P210 KI construct; the Flow Cytometry Facility (Department of Immunology, Karolinska University Hospital, Stockholm, Sweden) for collecting umbilical cord blood samples; and Kjell Sjöström (Innovogen, Lund, Sweden) for being very helpful in the generation of the KMT-1 antibody. This study was supported in part by the Swedish Cancer Society, the Swedish Children’s Cancer Foundation, the Swedish Cancer research Council (personal project to W.M.) and the Hemato-Linne and BioCare strategic program grants, the Medical Faculty of Lund University, the Swedish Society for Medical Research, and the IngaBritt and Arne Lundberg Foundation.