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Highlights

- BCR::ABL1 induces in the *Prom*^{-/-} background frequently an ALL phenotype.
- In humans, CML does not express PROM-1 on the surface whereas Ph+ALL does.
- Therapy resistance in Ph+ALL is associated with low PROM-1 expression.
- Targeting PROM-1 increases resistance, its expression augments sensitivity to TKIs.

Prominin-1 (CD133) is essential for the determination of the phenotype of Philadelphia chromosome-positive leukemia

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Key words: Philadelphia-chromosome (Ph⁺)-positive leukemia, Chronic Myeloid Leukemia (CML), Acute Lymphoblastic Leukemia (ALL), BCR::ABL1, cellular fate.

Abstract

The human PROMININ-1 (PROM-1, CD133), detected by its AC133 epitope, is expressed on the surface of leukemia-initiating cells of Philadelphia chromosome-positive (Ph⁺) leukemia cells. Ninety-five percent of chronic myeloid leukemia (CML) cases are Ph⁺. Ph⁺ acute lymphoblastic leukemia (ALL) accounts for twenty-five to thirty percent of adult ALL and is considered a high-risk ALL subgroup. Targeting the t(9;22)-fusion protein BCR::ABL1 with ABL1-directed kinase inhibitors (TKIs) is a proven therapeutic concept for CML and Ph⁺ ALL. Resistance against TKI comprises mutations in BCR::ABL1, but many patients exhibit a primary resistance with unknown mechanisms. Here, we investigated the impact of PROM-1 on the leukemogenesis

and the therapy response of Ph⁺ leukemias. We applied flow cytometry, co-immunoprecipitation, immunoblotting, stem cell assays, and leukemia induction *in vivo* to cell lines, syngeneic mouse models, and human Ph⁺ long-term cultures. We found a direct relationship between BCR::ABL1 and PROM-1, a shift from CML towards ALL upon the loss of Prom-1, as well as a link between the response to TKIs and the PROM-1 expression. In contrast to solid tumors, resistance was accompanied by a progressive loss of PROM-1. Our data suggest that PROM-1 plays an essential role in both cell fate decision and induction of the BCR::ABL1-related leukemic phenotype, highlighting its impact on the drug response of Ph⁺ leukemias.

Introduction

The Philadelphia chromosome-positive (Ph⁺), the der22 of t(9;22), is present in 95% of chronic myeloid leukemia (CML) and 25-30% of adult acute lymphoblastic leukemia (ALL) cases. It encodes the BCR::ABL1 fusion protein [1]. The SRC-related ABL1 fused to BCR leads to the constitutive activation of the ABL1 kinase [1]. Wild-type (wt) ABL1 is tightly regulated by a variety of stimuli. In contrast, constitutively activated ABL1 induces aberrant proliferation by activation of RAS protein, phosphatidylinositol-4,5-bisphosphate 3 (PI3) kinase, as well as JAK/STAT pathways [2]. The BCR::ABL1 expression in the murine bone marrow (BM) induces mainly a CML-like disease [3]. The suppression of the BCR::ABL1 kinase reverts disease induction [4, 5].

Inhibition of the BCR::ABL1 using inhibitors directed against ABL1-kinase (TKI) is the causal therapy of chronic phase CML (CP-CML) and essential to treating Ph⁺ ALL. Examples are ATP competitors such as imatinib, nilotinib, dasatinib, bosutinib, and ponatinib [6]. These compounds cannot eradicate the leukemic stem cell population and, thereby, the malignant clone, the source of relapse. The allosteric ABL1 inhibitor asciminib allows combining two ABL1 inhibitors [7].

Resistance to TKI is the primary clinical challenge for Ph⁺ leukemia. It is mainly caused by the selection of subclones expressing BCR::ABL1 harboring point mutations in the kinase domain [8]. However, in about 30% of drug-resistant patients, the detection of putative mutation in BCR::ABL1 has not been successful, and therefore, the mechanism of resistance remains unclear [9].

Although it is known that Ph⁺ leukemia expresses PROMININ-1 (PROM-1, alias CD133), its role in the response to treatment remains elusive [10]. PROM-1 belongs to the prominin family of pentaspan membrane glycoproteins [11, 12]. Human PROM-1 carrying the AC133 epitope is widely used as a surface antigen of hematopoietic stem and progenitor cells (HSPCs) [13, 14] as well as for the identification and isolation of stem and progenitor cells from various other tissue and organ systems (reviewed in [15]). Interestingly, human

CD34⁺ HSPC subpopulation expressing PROM-1/AC133 was shown to exhibit a high content of myeloid colony-forming cells [13, 16-18]. Murine BM-resident hematopoietic stem cells (HSCs) and myeloid precursors express Prom-1 [19, 20]. Although Prom-1 is dispensable for the pool size and function of mouse HSPCs during steady-state hematopoiesis, it modifies the growth-factor response of progenitor cells after myelotoxic stress, as demonstrated in *Prom-1*-knockout mice [19, 21].

At the molecular and cell biological levels, the main characteristics of PROM-1 can be summarized as follows:

i) it plays a vital role in the organization of plasma membrane protrusions such as those found in HSPCs by binding to the membrane cholesterol in cholesterol-based membrane microdomains [20, 22-26]; ii) it associates with extracellular vesicles (EVs) such as exosomes and ectosomes/microvesicles that are released in various body fluids [27]; iii) it is released from HSPCs in association with exosomes during the differentiation process [24]; iv) it is engaged in the PI3K/AKT pathway through its phosphorylation at the cytoplasmic tyrosine (TYR) 828 (numbered according to splice variant s2) by SRC family kinases SRC and FYN [28, 29]; v) it is involved in cell proliferation, differentiation, migration, drug resistance, intercellular communication and autophagy by being a part of the signaling through Wnt/b-catenin, TGF- β /Smad, MAPK/ERK and SRC-FAK pathways (reviewed in [15]).

Cancer-initiating (or stem) cells of several types of malignant tumors are positive for CD133 [30], and there is a relationship between CD133-positivity and therapy response. Notably, PROM-1/AC133-positivity confers resistance against cytostatic agents and radiotherapy to cancer cells [31].

Acute myeloid leukemia (AML), chronic myeloid leukemia (CML), and acute lymphoblastic leukemia (ALL) also express PROM-1/AC133 [32, 33]. Until now, limited information is available concerning the impact of PROM-1 and leukemogenesis and its relation to BCR::ABL1. Here, we investigated the role of PROM-1 in the determination of the leukemic phenotype given by leukemogenesis and response to TKIs mediated by BCR::ABL1. To this end, we used several cell lines and primary BCR::ABL1 models, including Ph⁺ long-term cultures (LTCs) and syngeneic BCR::ABL1 models in the presence or absence of Prom-1.

Our investigation reveals that PROM-1 is instrumental in cell fate decision and induction of the BCR::ABL1-related leukemic phenotype, underscoring its relevance to the drug response in Ph⁺ leukemias.

Materials and Methods

Cells and culture conditions.

Phoenix ecotropic cells and 293T cells were cultured in Dulbecco's modified Eagle medium (DMEM; Sigma-Aldrich) supplemented with 10% fetal calf serum (FCS; Gibco, Karlsruhe, Germany). The SupB15WT cells were obtained from the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany) and maintained in Gibco RPMI 1640 (Thermo Fisher Scientific) containing 15% FCS (Gibco). The SupB15RT cells were generated from the SupB15WT cells upon long-term exposure (3 months) to imatinib (1 μ M). Ph⁺ LTCs HP, PH, and BV) were previously described [33]. Imatinib and nilotinib were purchased from Selleckchem (Cologne, Germany).

Plasmids and short hairpin RNAs

The cDNA encoding the p185^{BCR::ABL1} has been previously described [34]. All retroviral expression vectors used in this study were based on the PINCO vector as previously described [36]. The PROM-1 cDNA derived from pCR3.1-uni-AC133 plasmid [14] was subcloned into Gateway[®]entry-vector (pENTR1A) for recombination into destination Gateway[®]vectors (retroviral PINCO and lentiviral pSIEW) according to the manufacturer's instructions (Invitrogen, Life Technologies). Lentiviral vectors expressing short hairpins (sh) RNA against human PROM-1 and non-targeting control (NTC, negative control - sc) were purchased from Sigma-Aldrich (Steinheim, Germany) as predesigned shRNAs and were directed against the following target sequences (italicized in the shRNA sequence): sh-PROM1-1: GCGTCTTCCTATTCAGGATAT, 5' -CCG *CGT CTT CCT ATT CAG GAT ATC* TCG AGA TAT CCT GAA TAG GAG ACG CTT TTT G-3'; sh-PROM1-2: GTTGAAACTATACCCATGAAA, 5'-CCG *GGT TGA AAC TAT ACC CAT GAA ACT CGA GTT TCA TGG GTA TAG TTT CAA CTT TTT* G-3'; sh-PROM1-3: GCAACAAATTATGAGACCCAA, 5'-CCG *GGC AAC AAA TTA TGA GAC CCA ACT CGA GTT GGG TCT CAT AAT TTG TTG CTT TTT* G-3'. sh-PROM1-4: CCCAACATCATCCCTGTTCTT, 5'-CCG *GCC CAA CAT CAT CCC TGT TCT TCT CGA GTTGGG TCT CAT AAT TTG TTG CTT TTT* G-3'; sh-PROM1-5: CCTCTGTTATTATTGAGGAAA, 5'-CCG *GCC TCT GTT ATT ATT GAG GAA ACT CGA GTT GGG TCT CAT AAT TTG TTG CTT TTT* G-3'.

Real-time PCR

RNA was isolated from SupB15 (WT and RT) and LTCs (HP, PH, and BV) using the RNeasy kit, while cDNA was synthesized using a cDNA synthesis kit according to the manufacturer's protocol (Qiagen, Düsseldorf, Germany). 2 μ l of PROM-1 template was used in 25 μ l reaction mixtures consisting of primers CD133-RT-PCR-F (GAAGAGCTTGCACCAACAAA) and CD133-RT-PCR-R (AGATGACCGCAGGCTAGTTT), both at 200

nM, and the breakpoint-specific probe CD133-probe-FAM(TCACGCTGGTCAGACTGCTGCT) labeled with FAM at the 5' end and TAMRA at the 3' end at 100 nM final concentration. qPCR Master Mix plus no rox sample was used with 5 mM MgCl₂ according to the supplier's instructions (Eurogentec, San Diego, USA). TaqMan® PCR was conducted in triplicate on an ABI PRISM 7700 (Applied Biosystems, Weiterstadt, Germany). The standard conditions were 1 cycle at 95°C for 10 min, 50°C for 2 min, and 72°C for 1 min, followed by 45 cycles at 95°C for 15 sec, 60°C for 1 min, and 72°C for 1 min. Normalization to ABL1 was done for each sample [37]. The CT values were exported into a Microsoft Excel worksheet to calculate fold changes using the Comparative CT method. The target amount normalized to an endogenous ABL1 is given by $2^{-\Delta\Delta CT}$.

Cell viability and proliferation

Cell viability and proliferation were assessed using the XTT proliferation kit according to the manufacturer's instructions (Roche, Mannheim, Germany).

Flow cytometry

All cells were washed twice in PBS and resuspended in PBS containing 1% bovine serum albumin (BSA; Sigma-Aldrich). Aliquots of 100 µl-cell suspensions were incubated for 30 min at 4°C with primary antibodies (Abs). The following primary Abs were used: AC133 PE-conjugated (Miltenyi Biotec, Bergisch Gladbach, Germany), anti-Sca1 and anti-c-Kit (BD Biosciences). After washing with PBS we analyzed 2×10^6 events on a FACSCanto II flow cytometer (BD Biosciences). The forward scatter channel (FCS) and side scatter channel were used to gate living cells followed by labelling with the lineage antibody cocktail (Miltenyi) and gating on lineage-negative cells. All instrument settings and gating strategies were established using isotype/secondary antibody controls. Data were analyzed using FlowJo (TreeStar, Ashland, USA) and FCS Express 7 software (De Novo Software). Median fluorescence intensity (MFI) was calculated as a difference in MFI values of stained and negative (iso-type/secondary Abs) populations.

Transfection, retroviral, and lentiviral infections

Transfection was performed using calcium phosphate precipitation following widely used protocols. Retro- and lentiviral supernatants from transfected Phoenix Ecotropic cells and 293T cells were obtained as described [38]. Target cells (1×10^5) were plated onto retronectin-coated non-tissue culture-treated 24-well-plates (Takara-Shuzo, Shiga, Japan), exposed to the retroviral or lentiviral supernatants and then centrifuged at 2.200 rpm for 45 min at 32°C. The infection was repeated thrice, and transduction efficiency (min. 70%) in both retro-

and lentivirus-infected cells was measured after 48 hrs by detecting enhanced green fluorescent protein (EGFP)-positive cells by flow cytometry.

Competition-proliferation assay

For the modified competition-proliferation assay (CPA) [38], cells (SupB15 or BV-LTCs) were infected with lentiviral pSIEW vectors harboring either shRNA or PROM-1 and co-expressing EGFP. Cells were exposed to 0.125-2 μ M or 0.1-5-5 μ M imatinib for 3 days, respectively. EGFP (and PROM-1) expression at day 1 was used to normalize their overall expression levels. CPA was performed by monitoring the EGFP expression on day 3 using flow cytometry.

Immunoblotting

Cells were washed in ice-cold PBS and then solubilized with lysis buffer (50 mM HEPES, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 25 mM NaF, 0.01 mM ZnCl₂, pH 7.0) containing 1% (v/v) Triton X-100 and 10% (v/v) glycerol (Roth, Karlsruhe, Germany) and supplemented with a protease inhibitor cocktail (Sigma-Aldrich) for 30 min at 4°C. The cell lysates were then recovered by centrifugation at 20,000 x *g* for 25 min. According to widely established protocols, proteins were separated by 4-12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The following Abs were used: anti-ABL1 (clone 24-11, Santa Cruz Biotechnology), anti-phospho-Y245 ABL1 (p-cAbl-Y245, Cell Signaling, Boston, USA), anti-PY (phosphotyrosine) (clone 4G10, Millipore), anti-PROM-1(clone 80B258) [39] and anti- α -tubulin (clone DM1a, Sigma-Aldrich) followed by appropriate peroxidase-conjugated secondary Abs. Blocking and incubation were performed in 5% low-fat dry milk, followed by washing in Tris-buffered saline (TBS) (10 mM Tris-HCl, 150 mM NaCl, pH 8.0) containing 0.1% Tween-20 (TBS-T). Membranes were incubated with enhanced chemiluminescence reagents (ECL system, GE Healthcare) and, afterward, developed either by the Odyssey imaging system (LICOR bio) or exposed to films (GE Healthcare) and quantified after digitization using the ImageJ software.

Co-immunoprecipitation

Cells were washed in ice-cold PBS and solubilized with lysis buffer (see above) supplemented with a protease inhibitor cocktail. After centrifugation at 20,000 x *g* for 25 min, supernatants were pre-cleared. Co-immunoprecipitation was performed overnight by incubating with CD133/1 monoclonal (m) Ab (clone AC133) and protein A/G-coupled agarose beads (Santa Cruz Biotechnology, USA) at 4°C, followed by six washing

steps in lysis buffer. Bound proteins were eluted by boiling samples in an SDS-PAGE loading buffer. Input proteins (50 g) and bound fraction materials (5.0 g) were separated on a 4-12% SDS-PAGE gel and probed sequentially by immunoblotting.

Differential centrifugation

PDLTCs-PH were treated and not with imatinib (1 μ M) for 1 month. Then, media conditioned for 24 hrs were collected and subjected to differential centrifugation as follows: 5 min at 300 x g, 20 min at 1200 x g, 30 min at 10,000 x g, and 1 hr at 200,000 x g. The resulting 200,000g pellets were re-suspended in Laemmli buffer and analyzed by immunoblotting.

Animals

All animal work was done under the license granted to Martin Ruthardt (F39/08-Regierungspräsidium Darmstadt). C57BL/6 mice were obtained from Janvier Labs (St.Berthevin, France), while *Prom-1^{-/-}* null mice were previously generated in the laboratory of Peter Carmeliet (KU Leuven, Leuven, Belgium) [21]. The animals were kept in the animal facility of the Medical Faculty of the Johann Wolfgang Goethe University on sterile bedding in filter-top cages (EBECCO). They were provided with sterile food (SSNIF- spend diet) and access to water ad libitum.

Isolation of murine hematopoietic stem and progenitor cells (HSCP)

Sca1⁺/lin⁻ HSPCs were isolated from 8–12-week-old animals (all females) after euthanasia by CO₂ asphyxia. BM was harvested from the femur and tibia by flushing the bones with a syringe using a 26-gauge needle. Cells were “lineage depleted” by labeling them with biotin-conjugated lineage panel Abs against B220, CD3 ϵ , Gr-1, Mac-1, and Ter-119 (Miltenyi Biotec and Stem Cell Technologies, Vancouver, Canada), followed by labeling with anti-biotin mAbs conjugated to magnetic microbeads (Miltenyi Biotec). The labeled cells were retained using the MACS cell separation columns according to the manufacturer’s instructions (Miltenyi Biotec). Sca1⁺ cells were purified using a mouse Sca1 positive selection kit (Stem Cell Technologies). The purified cells were pre-stimulated for 2 days in DMEM supplemented with 10% FCS (Hyclone/Perbio Science, Bonn, Germany), 1% L-Glutamine, 1% Penicillin/Streptomycin, mIL-3 (20 ng/mL), mIL-6 (20 ng/mL) and mSCF (100 ng/mL) (all cytokines from Cell Concepts, Umkirch, Germany). Two days later, cells were retrovirally transduced as described below.

Syngeneic mouse model of leukemia

8–12-week-old wt C57BL/6 (*Prom-1^{+/+}*) and C57BL/6-*Prom-1^{-/-}* mice were used as donors. Sca1⁺ cells were isolated and stimulated as described above. We irradiated C57BL/6 recipients sub-lethally with 4.5 Gy and inoculated them with 1x10⁵ cells transduced with empty (control) and BCR::ABL1 harboring vectors. Sca1⁺ cells were inoculated via the tail vein. Mice were sacrificed at the first appearance of morbidity, loss of weight (10%), neurological abnormalities, failure to thrive, or diarrhea over a period of 100 days. Whole BM and spleen cells were cytopun and stained with May-Grünwald-Giemsa stain for morphological analysis, while the expression of Gr-1, Mac-1, CD3e, and B220 markers (BD Pharmingen) was analyzed by flow cytometry. Student's t-test was used to determine the statistical relevance.

Colony-forming units – Methylcellulose assay

At day 5 post-infection, Sca1⁺ or Sca1⁺/lin⁻ cells were plated at 5x10³ cells/mL in methylcellulose supplemented with mIL-3 (20 ng/mL), mIL-6 (20 ng/mL) and mSCF (100 ng/ml). On day 10, the number of colony-forming units (CFUs) was determined. After washing out from the methylcellulose, cells were stained with specific Abs to detect surface marker expression by flow cytometry. Differentiation status was assessed by the expression of the surface markers c-Kit, Sca1, Gr-1, and Mac-1 (BD Pharmingen).

Colony forming unit – spleen day12 (CFU-S12)

Sca1⁺/lin⁻ cells isolated from *Prom-1^{+/+}* and *Prom-1^{-/-}* C57BL/6 mice were retrovirally transduced with BCR::ABL1 and cultured in DMEM medium supplemented with 10% FCS (Hyclone/Perbio Science), mIL3, mIL6, and mSCF for 7 days. Afterward, cells were harvested, intensively washed with PBS, and inoculated via the tail vein into lethally irradiated (11Gy) mice. Animals were sacrificed 12 days after inoculation, spleens were fixed in Tellysniczky's fixative, and the number of spleen colonies was determined as described elsewhere [40].

Statistical analysis

All statistical analyses were performed using Student-t-Test in GraphPad Prism 5 (GraphPad, Boston, Boston, MA, USA) software. $p \leq 0.05$ was considered significant. The data were presented as stated in the figure legends either as mean values from three independent experiments with corresponding standard deviation ($\pm$ SD) or as mean values of a representative performed in triplicate with corresponding standard error of the mean ($\pm$ SEM).

Results

Loss of Prom-1 in normal and malignant hematopoiesis

It has been shown that the lack of Prom-1 impacted the cytokine-sensitive progenitors in mice [19] and to study the impact of Prom-1 on normal hematopoiesis in comparison to malignant conditions, we ectopically expressed BCR::ABL1 fusion protein in murine Sca1⁺/lin⁻ HSPCs purified from wt (Prom-1^{+/+}) and Prom-1-deficient (Prom-1^{-/-}) C57BL/6 mice. The HSPCs derived from the two genetic backgrounds are referred to as Prom-1^{+/+} and Prom-1^{-/-} HSPCs, respectively. We infected the cells either with the PINCO empty vector (control) or the PINCO-BCR::ABL1 vector. At least 70% of cells expressed EGFP, the PINCO-associated reporter gene, as evaluated by flow cytometry (data not shown) [3].

First, we investigated the influence of Prom-1 background on the normal and BCR::ABL1-positive malignant hematopoiesis after 48 hrs from infection. To assess their viability and proliferation, we plated three concentrations of cells (2.500, 5.000, and 10.000 cells in 1 mL) in 24-well plates and performed an XTT assay. In the presence of cytokines (mIL3, mIL6, and mSCF), no difference was observed in Prom-1^{+/+} HSPCs independently of the BCR::ABL1 expression. In contrast, a significant reduction in the proliferation of BCR::ABL1-expressing Prom-1^{-/-} HPSCs was observed compared to the control, irrespective of the cell concentration used (Figure 1A, left panel). As expected, the absence of cytokines almost suppressed normal hematopoiesis, regardless of the presence or absence of Prom-1. At the same time, the BCR::ABL1-dependent proliferation of malignant cells was reduced in Prom-1^{-/-} HSPCs (Figure 1A, right panel).

Second, we performed a methylcellulose-based CFU assay to assess HSPCs' capacity to differentiate, and their ability to form colonies. In the presence of cytokines, the number of colonies is significantly reduced in Prom-1^{+/+} HSPCs expressing BCR::ABL1 compared to control cells. In contrast, an increased number of colonies was observed when BCR::ABL1 is expressed in Prom-1^{-/-} HSPCs, suggesting that Prom-1 itself has a negative impact on cytokine-driven differentiation in malignant hematopoiesis (Figure 1B, left panel). In agreement with this observation, the withdrawal of cytokines equalized the number of CFUs induced by BCR::ABL1 independently of the presence of Prom-1 (Figure 1B, right panel), suggesting that Prom-1 had a negative effect on the cytokine-driven differentiation in these cells.

Third, we performed a CFU-S12 assay using transduced HSPCs cultured for seven days in the presence of cytokines, which will be instructive on the influence of Prom-1 on BCR::ABL1 stem cell activity. Surprisingly, there was a significantly higher number of CFU-S12 in empty-vector (control) transduced Prom-1^{-/-} HSPCs than in Prom-1^{+/+} ones. There was a slight increase of CFU-S12 from Prom-1^{-/-} HPSC concerning Prom-1^{+/+}

HPSC expressing BCR::ABL1, albeit not statistically relevant (Figure 1C). These data were in line with the negative impact of Prom-1 on the cytokine effect observed in vitro. This event occurs in early HSPCs, as confirmed by a higher number of Sca1⁺/c-Kit⁺ HPSC with a Prom-1^{-/-} background in both normal and BCR::ABL1-positive hematopoiesis revealed by the CFU-S12 (Figure 1D). Altogether, these findings suggested an involvement of Prom-1 in regulating normal and malignant hematopoiesis.

A shift from CML-like disease towards ALL accompanies Prom-1 deficiency

Our findings that BCR::ABL1 influences stem and progenitor cells depending on the *Prom-1* background prompted us to investigate the Prom-1's role in the initiation and maintenance of BCR::ABL1-positive leukemia. Thus, we compared BCR::ABL1-positive syngeneic leukemias using Sca1⁺/lin⁻ HSPCs isolated from a *Prom-1*^{-/-} or a *Prom-1*^{+/+} genetic background [3].

Independently of the genetic background, no sub-lethally irradiated (4.5Gy) mice injected with cells harboring the empty vector (control) developed malignant disease over 100 days, suggesting that the lack of Prom-1 per se did not induce a malignant phenotype (Figure 2A). Interestingly, the leukemia induction by BCR::ABL1 was as efficient independently of the presence of *Prom-1* (Figure 2A). However, BCR::ABL1 promoted mainly a CML-like disease (>80%) in the *Prom-1*^{+/+} background, where a typical splenomegaly (600-1200 mg) and blood picture characterized by mature granulocytes were observed (Figure 2B and C, respectively). In contrast, a lymphoblastic morphology typical for ALL was detected in HSPCs derived from *Prom-1*^{-/-} mice (Figure 2D). These findings were confirmed by specific marker analyses, with high expression of B220 detected in *Prom-1*^{-/-} leukemic cells, while high expression of Gr-1 and Mac1 was observed in *Prom-1*^{+/+} leukemic cells (Supplementary Figure 1A and B). Interestingly, re-transplantation was only possible for *Prom-1*^{-/-} BCR::ABL1-ALLs and not for *Prom-1*^{+/+} or *Prom-1*^{-/-} BCR::ABL1-CML-like diseases, confirming the differences in malignancy between the ALL and CML, both induced by BCR::ABL1 (Figure 2E and F)

Thus, our data suggest that the lack of Prom-1 increases the malignancy of the disease associated with BCR::ABL1 as shown by the switch towards a higher proportion of ALL.

Prom-1 influences the response to imatinib of c-Kit⁺ and Sca1⁺ progenitors

The data described above, and the fact that imatinib is known not to affect BCR::ABL1-positive primitive progenitors [41] motivated us to study whether Prom-1 was able to modify the response of BCR::ABL1-positive HSPCs to the drug.

Here, we exposed BCR::ABL1 transduced HSPCs isolated from *Prom-1*^{+/+} and *Prom-1*^{-/-} mice to imatinib for three days and then analyzed its impact on the expression of two stem and progenitor cell markers (c-Kit and

Sca1) by flow cytometry. As a control, we used untreated cells. While imatinib treatment slightly reduced the number of Sca1⁺/c-Kit⁺ progenitors in malignant HSPC population isolated from *Prom-1*^{+/+} animals, it greatly increased them in those isolated from *Prom-1*^{-/-} mice (Figure 3A).

These observations suggest that Prom-1 regulates the pool of progenitors in a malignant cell population, which could explain the reduced amounts of CFUs in the methylcellulose-based and CFU-S12 assays observed in HSPCs derived from BCR::ABL1 expressing *Prom-1*^{+/+} animals.

In humans, if not treated, CML-CP progresses into a therapy-resistant state of acute leukemia called blast crisis (BC). In about 30% of cases, CML-BC is lymphoblastic, and the rest is myeloid. Therefore, we wanted to know whether our findings in *Prom-1*^{+/+} and *Prom-1*^{-/-} mice were applicable to human CML, namely whether a lymphoblastic BC would not express PROM-1 on its surface, whereas a myeloid BC would be positive for CD133. As there is no myeloid BC LTC, we analyzed the following cell lines for their PROM-1 surface expression: K562 (myeloid BC), BV173 (lymphoblastic BC), and SupB15 (Ph⁺ ALL). The HCT116 colon cancer cell line was used as a PROM1-positive control. In addition, we compared two Ph⁺ LTCs from patients with lymphoblastic BC, CM, and VB, with two Ph⁺ (PH and BV) and one Ph⁻ (HP) ALL LTCs. PROM-1 surface expression (CD133 positivity) was assessed using an AC133 antibody compared with isotype IgG controls. As shown in Figure 3B, all BC models were CD133 negative, whereas all Ph⁺ ALL models were CD133-positive. Unlike the mouse models, by determining the CD133 expression, we could distinguish between human Ph⁺ ALL and CML-BC, but not between lymphoblastic and myeloid BC in CML.

Relationship between PROM-1 expression and sensitivity to TKIs in human Ph+ALL

Our previous findings showed a relationship between surface PROM-1 expression and response to TKI in human Ph⁺ models. Thus, we compared the PROM-1 expression in two human Ph⁺ ALL cell lines, SupB15WT and SupB15RT. SupB15RT cells were resistant to imatinib and other TKI [3].

Cell surface expression of PROM-1 was assessed by flow cytometry using the AC133 mAb. Interestingly, whereas SupB15WT expressed the PROM-1 protein on the surface, SupB15RT did not (Figure 4A).

As PROM-1 is released in association with small EVs during the process of HSPC differentiation [24], we investigated whether the reduction of cell-associated PROM-1 is related to its release. To this end, small EVs were enriched by differential centrifugation from conditioned media, and the 200,000g pellet was analyzed by immunoblotting. After probing with an anti-PROM-1 mAb, no corresponding 120-kDa immunoreactive band was detected in media collected from SupB15RT cells compared with those associated with SupB15WT cells, indicating that the reduction in PROM-1 is not related to PROM-1⁺ EV release (Supplementary Figure 2). The

expression of PROM-1 was then determined at the transcriptional level. RNAs were isolated from the two cell lines and analyzed by qPCR. We found that the reduction of PROM-1 corresponds to suppression of its gene expression (Figure 4B).

To confirm that down-regulation of PROM-1 is linked to the induction of imatinib resistance in SupB15WT cells, we monitored its expression after 1 and 3 months of drug exposure (0.5 μ M). Interestingly, SupB15WT cells showed a progressive reduction in PROM-1 expression over time to the level of previously established SupB15RT cells (Figure 4C). Moreover, the imatinib treatment did stimulate the release of PROM-1⁺ EVs (Supplementary Figure 2). As with imatinib, exposure to nilotinib reduced PROM-1, reinforcing the link between PROM-1 expression and drug resistance (Supplementary Figure 2b).

To get more insight into this phenomenon, we silenced PROM-1 in SupB15WT cells. Using a lentiviral-based system, we transduced four distinct shRNAs (sh1-4) against PROM-1. Their efficacy was evaluated by immunoblotting (Figure 4D). Sh2 and 4 caused the greatest reduction of PROM-1 compared to the unrelated control shRNA. We then used one weak (sh1) and one potent (sh2) suppressor of PROM-1. Interestingly, the proliferation of both sh1 and sh2 transduced SupB15WT cells was increased in the presence of imatinib in a dose-dependent manner (0-2 mM), compared to control shRNA, suggesting that the suppression of the PROM-1 expression increases the imatinib resistance (Figure 4E). Ph⁺ ALL and CML-CP are biologically distinct entities with different clinical behavior and treatment goals. However, if administered as single agent, the response rate of Ph⁺ ALL to TKI is lower than that of CML-CP patients although the addition of TKI to the therapy regimen improved the prognosis of Ph⁺ ALL.

Finally, we restored PROM-1 expression in SupB15RT cells to assess the positive effect of PROM-1 on the response to imatinib. We transduced them using retrovirally (PINCO) and lentivirally (pSiew) based systems. Transduced cells were then exposed to different concentrations of imatinib (0-1 μ M), and afterward, we performed a CPA following the EGFP expression by flow cytometry. In line with the data presented above, a reduction in the percentage of EGFP⁺ cells re-expressing PROM-1 was detected as dose-dependent. At the same time, no effect was observed with SupB15RT cells transduced with control empty vectors (Figure 4F). These data highlight a relationship between PROM-1 expression and response to imatinib in human Ph⁺ ALL cell lines consistent with data from mouse models.

Ph⁺ ALL and CML-CP are biologically distinct entities with different clinical behavior and treatment goals. However, if administered as single agent, the response rate of Ph⁺ ALL to TKI is lower than that of CML-CP patients although the addition of TKI to the therapy regimen improved the prognosis of Ph⁺ ALL. PROM-1 expression and the imatinib response in Ph⁺ long-term cultures

As the features of Ph+ ALL in adults are not fully reproduced in established cell lines, we decided to compare the PROM-1 expression in Ph+ LTCs closer to ALL than cell lines [42, 43]. Here, we used three distinct LTCs. The PROM-1 expression was evaluated by flow cytometry and qPCR in two LTCs, the fully TKI-sensitive PH and the fully resistant BV cells. In BV, the therapy resistance was not due to a mutation in BCR::ABL1 [3]. The Ph- LTC HP was used as a negative control.

TKI-sensitive LTC PH showed a high surface expression of PROM-1, whereas TKI-resistant LTC BV showed an expression that was as low as that of the Ph- LTCs HP (Figure 5A). qPCR confirmed that the expression of PROM-1 in LTCs was regulated at a transcriptional level (Figure 5B).

Although Ph+ ALL and CML-CP are biologically distinct entities with different clinical behavior and treatment goals they both respond to TKI administered as single agent. The response rate of Ph+ ALL to TKI is lower than that of CML-CP patients although the addition of TKI to the therapy regimen improved the prognosis of Ph+ ALL.

The relationship between PROM-1 expression and imatinib resistance was further dissected by silencing the PROM-1 expression in Ph+ LTC PH. Efficient targeting of PROM-1 was observed by immunoblotting, where sh2 and sh5 gave the best down-regulation (Figure 5C). Again, silencing PROM-1 reduced response to imatinib, as assessed by XTT assay, in a dose-dependent manner (Figure 5D). On the other hand, the overexpression of PROM-1 in TKI-resistant Ph+ LTC BV (Figure 5E, left panel) decreased their proliferation in the XTT assay, thereby reducing their imatinib-resistance (Figure 5E, right panel).

These findings confirmed the data we obtained in the SupB15WT and SupB15RT cells.

The interaction between PROM-1 and BCR::ABL1 appears independent on BCR::ABL1 phosphorylation.

The fact that PROM-1 and BCR::ABL1 interacted functionally, e.g., determining the response to TKI, prompted us to determine a potential physical interaction.

First, we performed a co-immunoprecipitation (Co-IP) on the Ph+ LTCs PH and BV and on Ph- LTCs HP as a control using AC133 mAb directed against PROM-1. The input and bound fraction were probed for BCR::ABL1 and PROM-1 using Abs against the ABL1 portion of BCR::ABL1 and PROM-1 (Figure 6A). No interaction between PROM-1 and ABL1 was observed in the three used cells. Interestingly, the BCR::ABL1 fusion protein is pulled down along PROM-1 in TKI-sensitive PH, a phenomenon hardly observed in TKI-resistant BV, where PROM-1 expression is low. Second, we investigated whether the phosphorylation of BCR::ABL1, which regulates its activity, impacted its interaction with PROM-1. Before the PROM-1 IP, TKI-sensitive PH cells were treated with TKI imatinib and nilotinib at various concentrations. Samples were then probed with Abs against phospho-ABL1-Y245, total ABL1, PROM-1, and phospho-tyrosine residue(s) (PY) (Figure 6). Interestingly, the

PROM-1-BCR::ABL1 interaction appeared not to be completely abrogated in the presence of imatinib (Figure 6B, left panel). Indeed, the slight reduction of unphosphorylated BCR::ABL1 in the bound fraction reflected its overall reduction as observed in the input for drug-treated samples. Furthermore, PROM-1 engaged in the BCR::ABL1 interaction was not phosphorylated. Similar data were obtained with nilotinib (Figure 6B, right panel).

Altogether, these data suggest that the effect of PROM-1 in BCR::ABL1-driven malignant hematopoiesis involves both drug response and cell fate decision. Thus, PROM-1 has an influence in the leukemogenesis of Ph⁺ leukemia.

Discussion

In this study, we uncovered the involvement of PROM-1 in the pathogenesis and response to targeted therapy of Ph⁺ leukemia. The expression of BCR::ABL1 in Sca1⁺ HSPCs derived from C57BL/6 mice leads to CML-like disease and rarely to BCR::ABL1-driven ALL. The CML-like disease is characterized by myeloproliferation and represents the best model available for human CML-CP [3]. The CML-like disease was not transplantable. Surprisingly, murine Prom-1 is involved in the cell fate decision of BCR::ABL1-driven hematopoietic disorders. The loss of Prom-1 reduced the number of CML-like diseases (not transplantable) and increased the number of BCR::ABL1-driven ALL (transplantable) induced by the expression of BCR::ABL1 in Sca1⁺ HSPCs. This shift from myeloproliferation towards acute leukemia represents an increase in malignancy and a reduction of response to targeted therapy.

No differences in progenitors were seen between *Prom-1*^{+/+} and *Prom-1*^{-/-} mice prior to the transduction of the Sca1⁺ HSPCs in agreement with a previous study [19]. However, an aberrant differentiation in *Prom-1*^{-/-} cells with increased lymphoblastic progenitors would explain a shift from CML-like disease versus ALL. Prom-1 seems required in mice for a regular differentiation of the myeloid progenitors as a reduced frequency of growth factor-responsive myelo-erythroid precursors was observed [19]. Thus, BCR::ABL1 may target more immature cells in a *Prom-1*^{-/-} background, which is consistent with *Prom-1*^{-/-} leading to an increase of immature HSPCs as shown by the CFU-S12, where the loss of Prom-1 increased the colony formation, independently of BCR::ABL1. Whereas in normal hematopoiesis, colony formation in CFU-S12 indicates the number of early progenitors of myeloid cells [44], in malignant hematopoiesis, it is an additional indicator for leukemia and the presence of leukemic stem cells [40, 44].

It seems that the action of imatinib on murine HSPC expressing BCR::ABL1 is inhibited by Prom-1. If this effect is due to i.) the difference between mouse and man, ii.) Specificity for the murine cell strain, or iii.) the increase in aberrant lymphatic differentiation, needs further investigation. It is further complicated by differences between murine and human hematopoiesis, as the mouse is characterized by physiological lymphocytosis.

In human solid tumors, high expression of PROM-1 is synonymous with a poor prognosis. PROM-1 is, therefore, a potential target for molecular therapy, particularly for cancer cells with stem cell properties [45]. In contrast, our data suggest that targeting PROM-1 in Ph⁺ diseases would decrease the response to targeted therapy by TKI. BCR::ABL1 might have different leukemic activity depending on the presence or absence of Prom-1. Inhibiting the constitutive kinase activity of BCR::ABL1 is a proven therapeutic concept in both CML and Ph⁺ ALL. Ph⁺ ALL and CML-CP are biologically distinct entities with different clinical behavior and treatment goals. However, if administered as single agents, the response rate of Ph⁺ ALL to TKIs is lower than that of CML-CP patients, although the addition of TKIs to the therapy regimen improved the prognosis of Ph⁺ ALL [46, 47]. The reason for this discrepancy between Ph⁺ ALL and CML-CP is yet to be understood.

In Ph⁺ ALL, there was a relationship between PROM-1 expression and their response to inhibition of BCR::ABL1 kinase by TKI. Resistant cells downregulated PROM-1 at a transcriptional level, while its forced overexpression restored the response to imatinib. These findings strongly correlate with results showing that imatinib decreased the proliferation of PROM-1⁺ cells without inducing apoptosis or interfering with the engraftment of cancer-initiating cells [48].

Mechanisms by which the PROM-1 may influence the therapy response of Ph⁺ leukemia cells are still elusive. The interaction between PROM-1 and BCR::ABL1 in a phosphorylation-independent manner could explain the role of PROM-1 in the response of Ph⁺ leukemia to TKI. However, there are differences between imatinib and nilotinib. regarding the transcriptional suppression of PROM-1 and their effects on PROM-1 expression, suggesting an additional effect of TKIs independent of the kinases targeted. Imatinib and nilotinib are both ABL1 ATP competitors and type II inhibitors [49, 50]. The crystal structure of imatinib bound to the kinase domain of ABL1 evidenced a conformation of the kinase where the activation loop is in a so-called "inactive" conformation (type-II binding)[51, 52]. Now its piperazine-benzamide part can access an additional hydrophobic pocket directly adjacent to the ATP binding site, and mimics a bound peptide substrate [53]. It has been shown that nilotinib is less susceptible to changes in the peptide conformation of the main target BCR::ABL1 than imatinib. Nilotinib is 20-30 times more potent than imatinib and active against some imatinib resistance mutants of BCR::ABL1 (54).

From our presented work, we can draw two conclusions: the first is that Prom-1 is involved in the cell fate decision of malignant hematopoiesis in mice, as its presence or absence is decisive for BCR::ABL1 to induce CML or ALL. The second is that the absence of PROM-1 increases drug resistance towards ABL1 inhibiting TKIs like imatinib or nilotinib in humans.

All these data suggest that PROM-1 is an important regulator of the cell fate decision and the rate of malignancy in Ph⁺ diseases and, thereby, of the response to targeted therapy.

These findings put PROM-1 at the center of the BCR::ABL1 biology and the phenotype of Ph⁺ leukemia.

Supplementary Materials: Figure S1: Expression of specific markers in BCR::ABL1 induced HSPCs isolated from *Prom-1*^{+/+} and *Prom-1*^{-/-} mice; Figure S2: Release of PROM-1 in association with extracellular vesicles; Acquisition of nilotinib-resistance is associated with a reduction of PROM-1 expression.

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Figure Legends

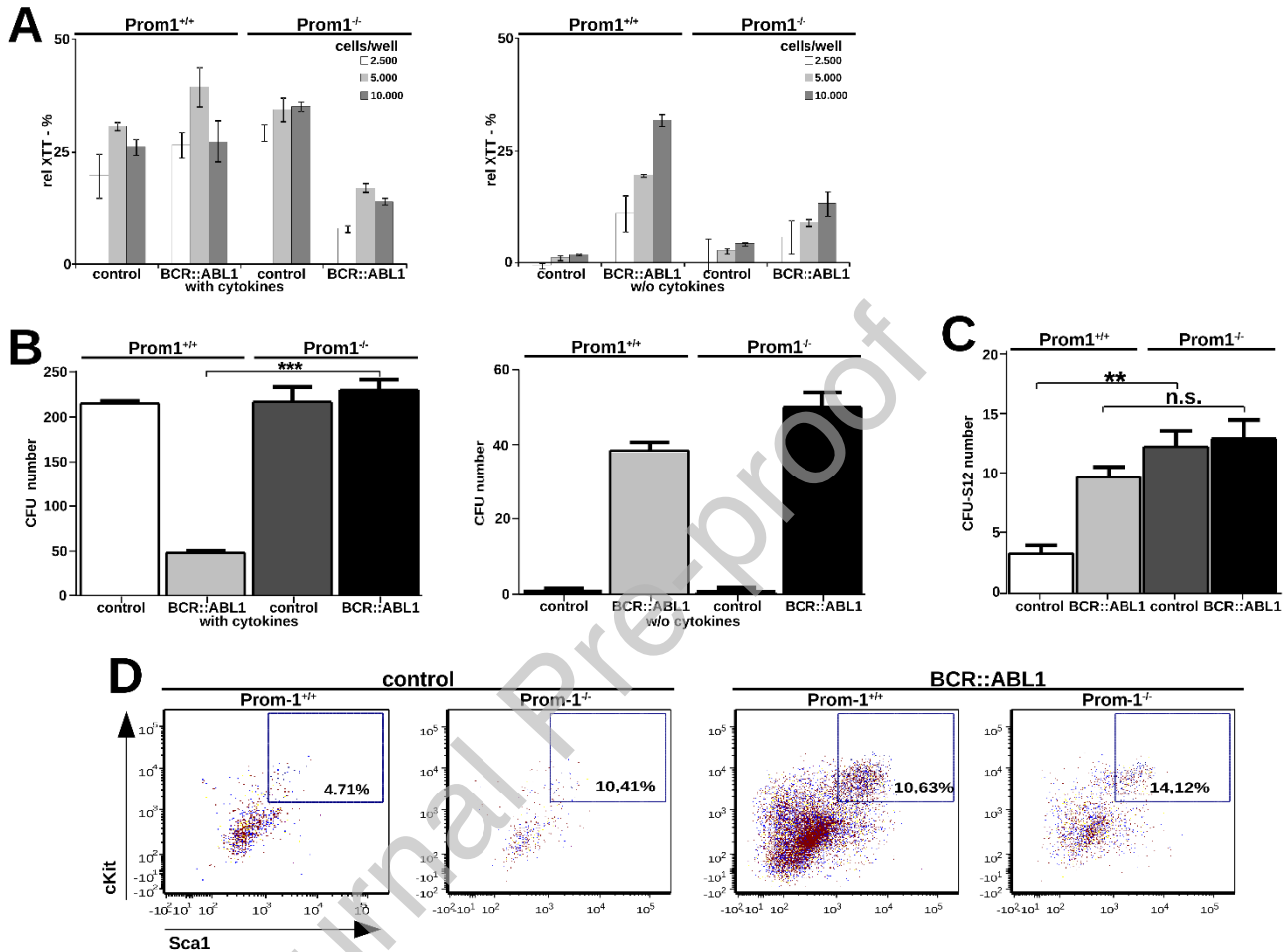


Figure 1. Significance of Prom-1 on normal and malignant hematopoiesis *in vitro* and *in vivo*. Sca1⁺/Lin⁻ HSPCs isolated from wt (*Prom-1*^{+/+}) or Prom-1-deficient (*Prom-1*^{-/-}) mice were transduced with empty (control) or BCR::ABL1-encoding PINCO-based retroviruses, and used as models of normal and malignant hematopoiesis. **A.** Cell proliferation was evaluated *in vitro* by an XTT assay at day 3. The data show a representative experiment of three carried out in triplicate. Mean $\pm$ SEM are presented. **B.** Colony formation was measured by methylcellulose-based CFU assays, supplemented with and without (w/o) cytokines, respectively. Three independent experiments and mean $\pm$ SD are presented. **C.** Transduced HSPCs indicated above were analyzed *in vivo* by a CFU-S12 assay. After 7 days in culture in the presence of cytokines, cells were transplanted into lethally (11Gy) irradiated recipient mice. Animals were sacrificed on day 12 after transplantation and the spleen colonies were counted. Each group comprised 4 mice. Mean $\pm$ SEM are presented. **D.** Expression of stem cell marker c-Kit and Sca1 on Prom-1^{+/+} or Prom-1^{-/-} in HSPCs transduced

either with an empty vector or with BCR::ABL1. We eliminated dead cells/debris in the FSC and SSC channels and considered the gated cells as living cells. Then lineage positive cells were excluded (see Materials and Methods) before gating cKit and Sca1 positive cells. One representative experiment is presented ($n = 3$). ** $p < 0.01$; *** $p < 0.001$.

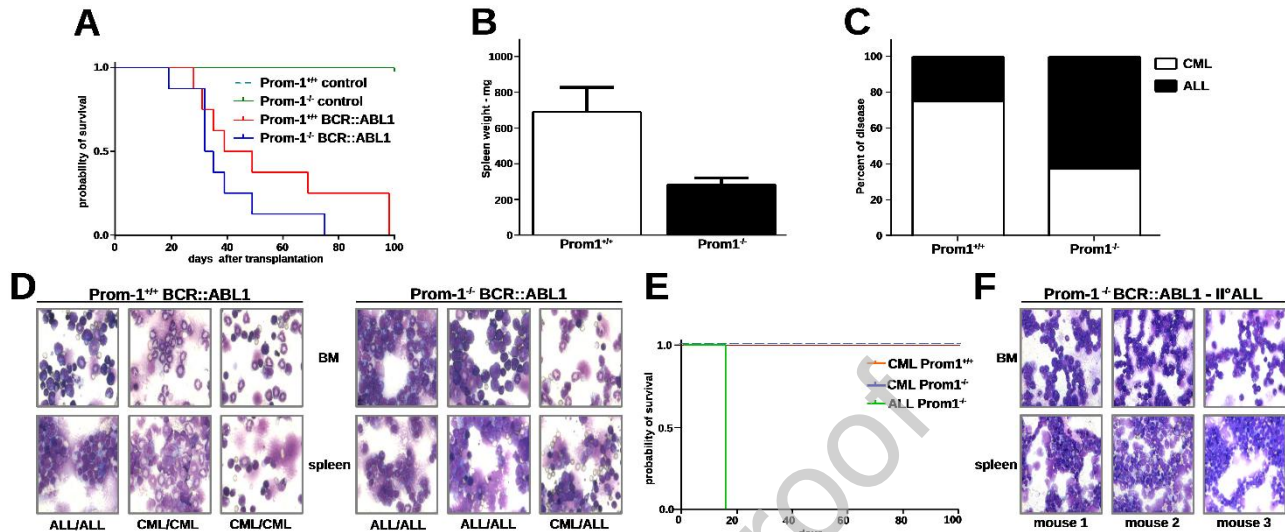


Figure 2: Prom-1 and BCR::ABL1 induced leukemogenesis. The transduction and transplantation model is based on Sca1⁺ cells isolated from wt (*Prom-1*^{+/+}) and Prom-1-deficient (*Prom-1*^{-/-}) mice. After BCR::ABL1 transduction, Sca1⁺ cells were transplanted into sub-lethally irradiated (4.5Gy) mice. **A.** Kaplan-Meier curve of survival within 100 days was established for each group ($n=8$ mice per group). **B.** Spleen weight of leukemic mice. **C.** Percentage of BCR::ABL1-induced ALL or CML in transplanted animals are presented. **D.** BM and spleen cells were cytopspun and stained with May-Grünwald-Giemsa. **E.** Spleen cells from ill mice, either CML (*Prom-1*^{+/+}) or ALL (*Prom-1*^{-/-}), were transplanted, and the Kaplan-Meier survival curves within 100 days were established for each condition ($n=3$ mice/group, left panel). **F.** BM and spleen from the three leukemic mice transplanted with (*Prom-1*^{-/-}) ALL were stained with May-Grünwald-Giemsa.

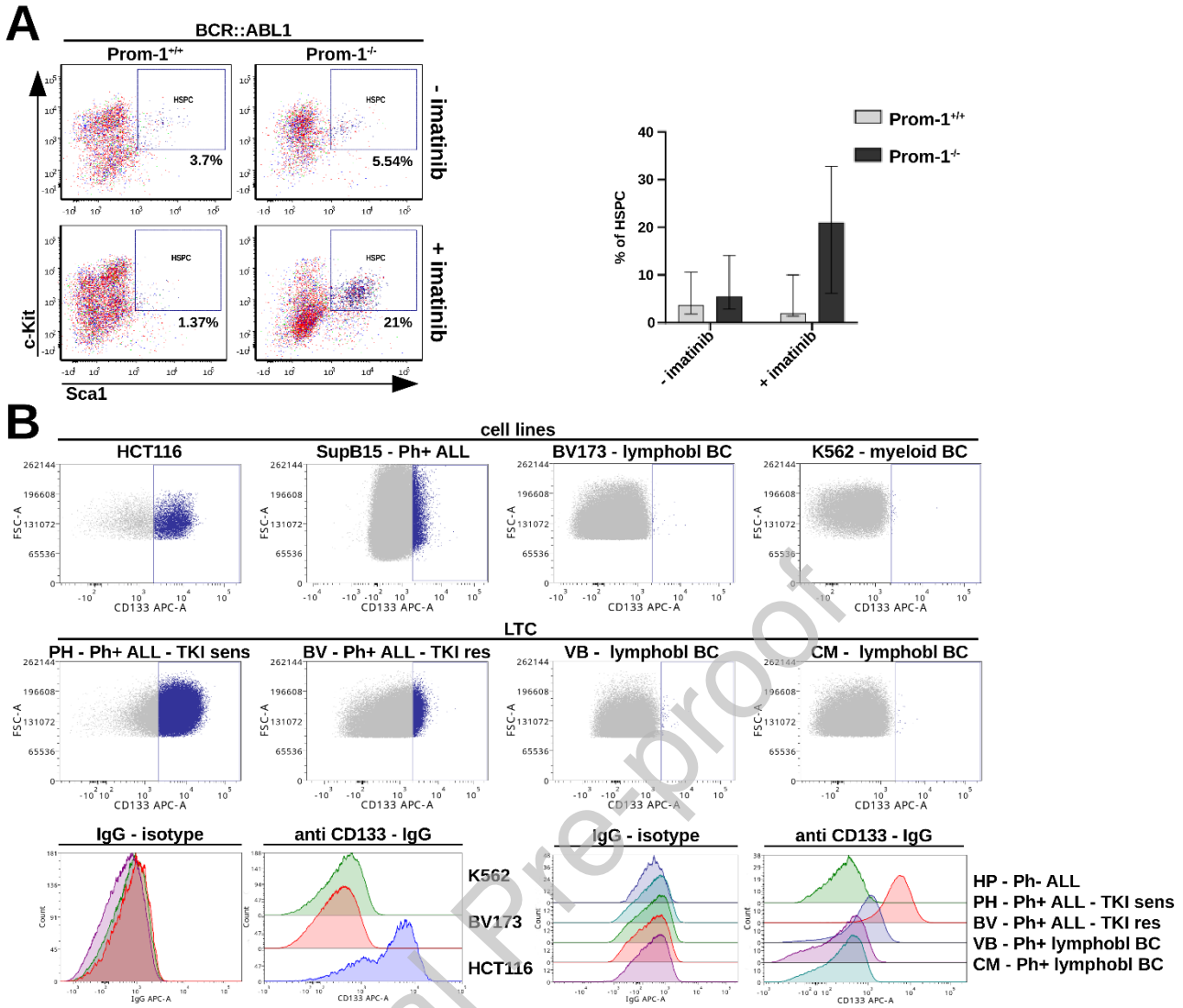


Figure3

Figure 3. A. Effects of imatinib on the stem cell population in BCR::ABL1-transduced HSPCs derived from *Prom-1*^{+/+} or *Prom-1*^{-/-} mice. Expression of c-Kit and Sca1 in murine *Prom-1*^{+/+} or *Prom-1*^{-/-} HSPCs transduced with BCR::ABL1 was evaluated by flow cytometry after three days of exposition to 1 μ M imatinib. Left panel - One representative experiment out of three performed is shown. We eliminated dead cells/debris in the FSC and SSC channels and considered the gated cells as living cells. Then lineage positive cells were excluded (see Materials and Methods) before gating cKit and Sca1 positive cells. Right panel - Median of three experiments (three biological replicates) with SD. **B. PROM-1 expression on the surface (CD133-positivity) of Ph+ ALL and CML-BC.** Cell lines: HCT116 - human colon cancer - positive control, SupB15 - Ph+ ALL, BV173 - lymphoblastic BC, K562 - myeloid BC. LTCs: HP - Ph- ALL - negative control, PH - Ph+ ALL - positive control, BV - Ph+ ALL TKI resistant, VB and CM - lymphoblastic BC.

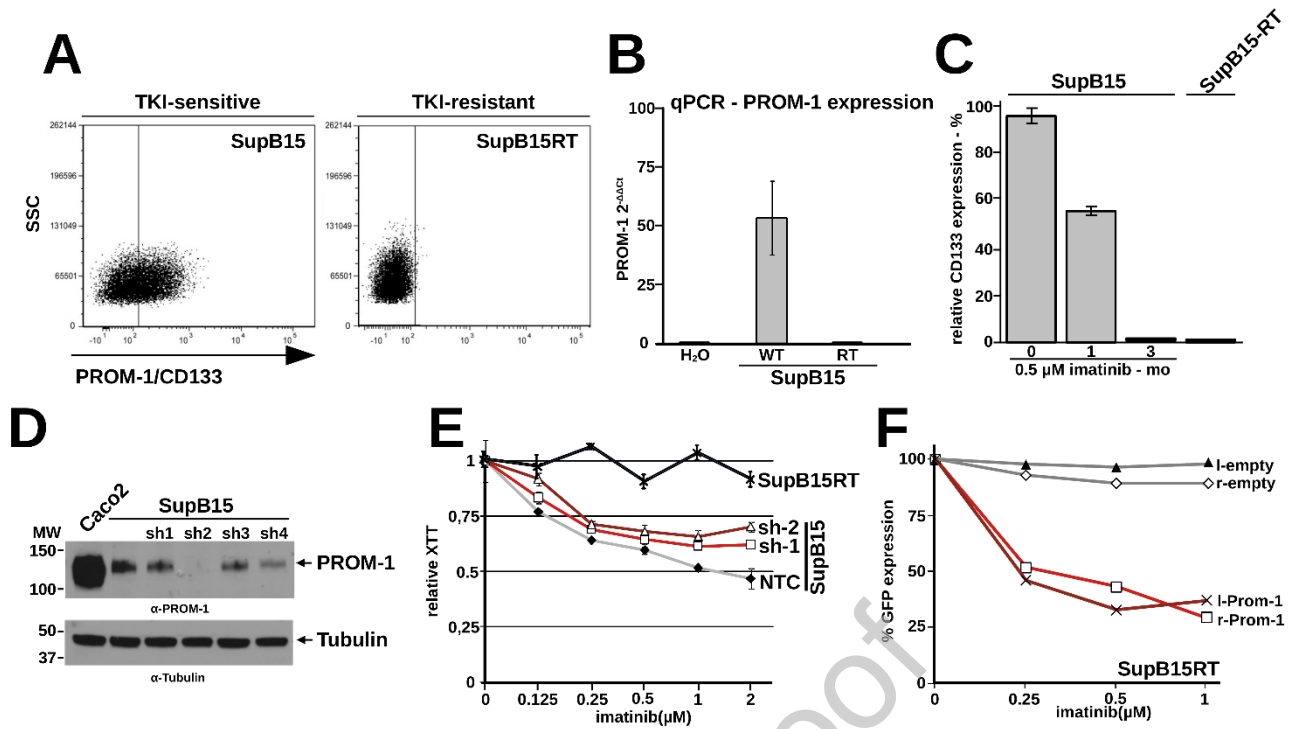


Figure 4. PROM-1 expression in imatinib-sensitive and resistant human Ph+ ALL cell lines. **A.** PROM-1 expression in SupB15WT (SupB15) and SupB15RT cells was evaluated by flow cytometry using PE-conjugated mAb AC133. SSC - side scatter channel. **B.** The relative concentration of PROM-1 mRNA was normalized to the concentration of the housekeeping gene ABL1 in qPCR. It is represented as $2^{-\Delta\Delta CT}$. One representative experiment performed in triplicate is presented (mean $\pm$ SEM, $n=3$). **C.** SupB15WT cells were exposed to imatinib (0.5 mM) for three months (mo), and PROM-1 expression was monitored by flow cytometry together with SupB15RT cells. Data represent the mean $\pm$ SD of three experiments. **D.** RNAi targeting PROM-1. As indicated, four distinct lentiviral expression vectors harboring shRNA (sh1-4) were used to suppress PROM-1 expression in Sup15WT cells. Immunoblotting against PROM-1/CD133. Caco-2 cells, positive control; α -tubulin, loading control. **E.** SupB15WT transduced with the sh1 and sh2 and an unrelated control shRNA (NTC). The transduced cells were then plated for 3 days with different concentrations of imatinib (0-2 μ M), and their proliferation was evaluated using XTT assay. SupB15RT cells were used as a positive control. Data represent the mean $\pm$ SD of three independent experiments. **F.** Competitive proliferation assay (CPA) for parental SupB15RT and cells transduced with an expression vector harboring PROM-1. SupB15RT cells were transduced retro- and lentivirally with empty vectors (PINCO and pSIEW, respectively) and vectors harboring PROM-1 and EGFP. The cells were treated with imatinib (0-1 μ M), and the EGFP expression was monitored by flow cytometry.

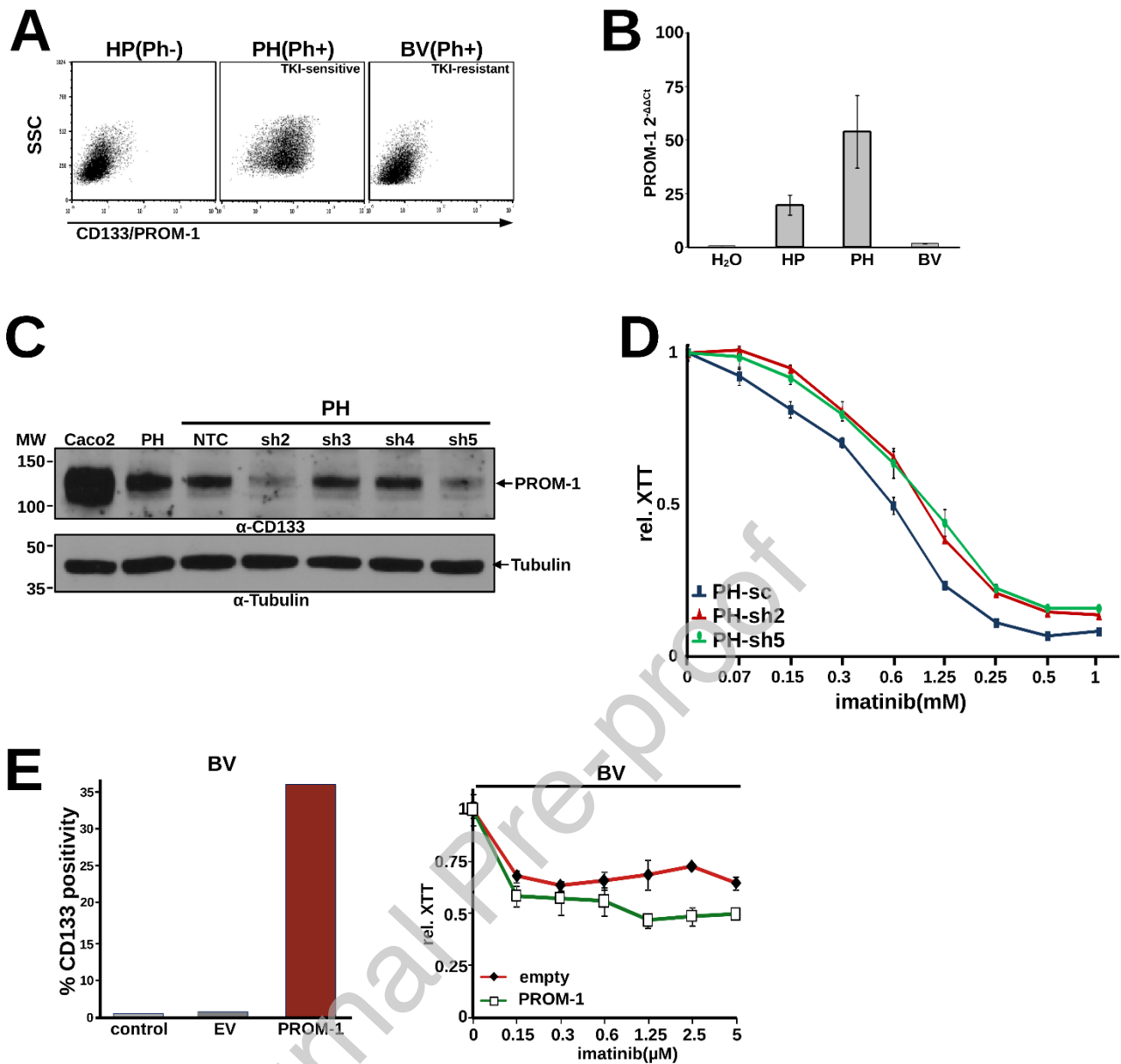


Figure 5. Effect of PROM-1 on the imatinib resistance in LTCs. PROM-1 expression in the LTCs HP (Ph-), PH (Ph+/TKI-sensitive), and BV (Ph+/TKI-resistance). **A.** PROM-1 expression in LTCs assessed by flow cytometry using the PE-conjugated AC133 mAb. SSC - side scatter channel; CD133/prom-1 - antibody used. **B.** Expression of the PROM-1 transcript by qPCR. The relative concentration of each mRNA was normalized to the concentration of the housekeeping gene ABL-1 and is represented as $2^{-\Delta\Delta CT}$. One representative experiment performed in triplicate is presented (mean $\pm$ SEM, $n=3$). **C.** Expression of PROM-1 in PD-LTCs-PH upon silencing using lentiviral expression-based shRNA (sh2-sh5). An unrelated shRNA (sc) was used as a negative control. PROM-1 was monitored by immunoblotting using an anti-PROM-1 Ab. An anti- α -tubulin Ab was used as a loading control. Human Caco-2 and untreated PH cells were loaded as positive controls. Apparent molecular weight markers are indicated. **D.** PH cells transduced with sh2 or sh5 and the control shRNA (sc) were then exposed for 3 days to different concentrations of imatinib (0-1 μ M). Their proliferation

was evaluated using an XTT assay. Data represent the mean $\pm$ SD of three independent experiments. **E.** Lentiviral transduction of PROM-1 in BV cells and response to imatinib. PROM-1 expression was monitored by flow cytometry using the PE-conjugated AC133 mAb (control, no treatment; ev, empty vector (left panel). PROM-1-transduced PD-LTCs-BV (PROM-1) or those infected with empty vector (empty) were exposed for 3 days with increasing concentrations of imatinib (0-5 μ M). Their proliferation was assessed by the XTT assay (right panel). Data represent the mean $\pm$ SD of three independent experiments.

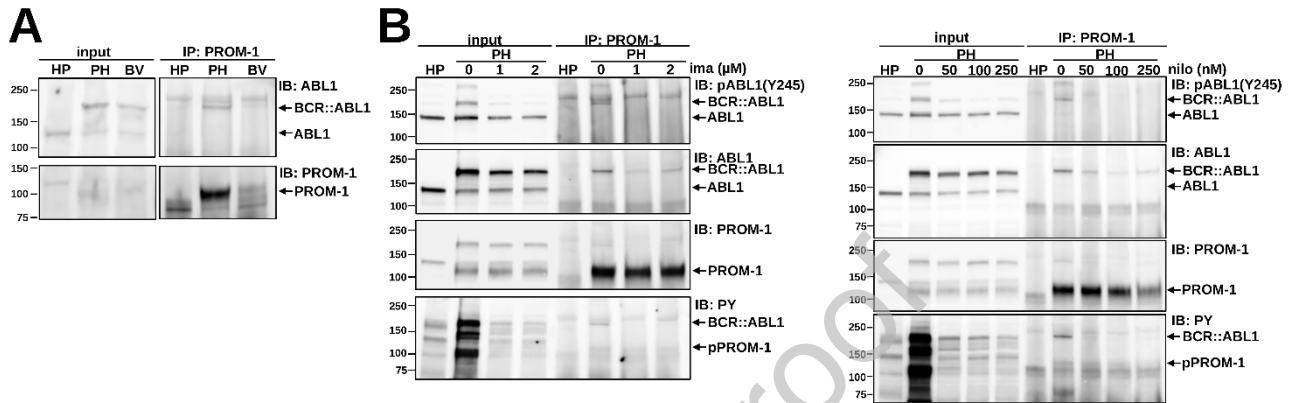


Figure 6. The interaction between PROM-1 and BCR::ABL1. A. Co-immunoprecipitation (IP) of PROM-1 and BCR::ABL1 in HP, PH, and BV cells. Cells were lysed and pre-cleared with protein A/G-agarose beads, and PROM-1 was immunoprecipitated using AC133 mAb and protein A/G-agarose beads and analyzed by immunoblotting using anti-PROM-1 and ABL1 Abs. Input and bound fraction (IP) were analyzed. **B.** Co-immunoprecipitation of PROM-1 and BCR::ABL1 in the absence/presence of imatinib or nilotinib (left and right panels, respectively). Blots were sequentially probed with anti-pABL1 (Y245), anti-ABL1, anti-PROM-1, and anti-PY (clone 4G10) Abs. Between each Ab incubation, blots were stripped. Apparent molecular weight markers are indicated.

Teaser abstract

The Philadelphia chromosome (Ph) is the product of the t(9;22), which fuses the *BCR* gene on chromosome 22 to the *ABL1* gene on chromosome 9. The result is the BCR::ABL1 oncogene, which is responsible for the induction of chronic myeloid leukemia (CML) and for about a third of acute lymphoblastic leukemia (Ph+ ALL). Only little is known about how t(9;22) induces either CML or ALL, and why there is a difference between the two diseases regarding their response to inhibitors that can inactivate BCR::ABL1. Here, we show that a gene called *PROMININ-1* is involved in both

processes: determining whether BCR::ABL1 induces CML or ALL, and explaining the weaker response of Ph+ ALL compared to CML to inhibitors of BCR::ABL1. We found that the reduction of PROMININ-1 increased the proportion of ALL induced by BCR::ABL1 in the mouse. We could not reproduce these findings in humans but distinguish CML from Ph+ ALL by the expression of PROMININ-1. Also resistance to BCR::ABL1 inhibitors was associated with low expression of PROMININ-1 in Ph+ ALL. These findings are in contrast to observations in solid tumors where a higher level of PROMININ-1 is accompanied by therapy resistance.

Our findings contribute to a better understanding of the different types of Ph+ leukemia and their response to therapy, which will help patients better manage these diseases.