CANCER

Tyrosine kinase inhibitor–induced CD70 expression mediates drug resistance in leukemia stem cells by activating Wnt signaling

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In chronic myelogenous leukemia (CML), oncogenic BCR-ABL1 activates the Wnt pathway, which is fundamental for leukemia stem cell (LSC) maintenance. Tyrosine kinase inhibitor (TKI) treatment reduces Wnt signaling in LSCs and often results in molecular remission of CML; however, LSCs persist long term despite BCR-ABL1 inhibition, ultimately causing disease relapse. We demonstrate that TKIs induce the expression of the tumor necrosis factor (TNF) family ligand CD70 in LSCs by down-regulating *microRNA-29*, resulting in reduced *CD70* promoter DNA methylation and up-regulation of the transcription factor specificity protein 1. The resulting increase in CD70 triggered CD27 signaling and compensatory Wnt pathway activation. Combining TKIs with CD70 blockade effectively eliminated human CD34$^+$ CML stem/progenitor cells in xenografts and LSCs in a murine CML model. Therefore, targeting TKI-induced expression of CD70 and compensatory Wnt signaling resulting from the CD70/CD27 interaction is a promising approach to overcoming treatment resistance in CML LSCs.

INTRODUCTION

Chronic myelogenous leukemia (CML) originates from leukemia stem cells (LSCs) harboring the constitutively active BCR-ABL1 tyrosine kinase (*1*). Imatinib, a tyrosine kinase inhibitor (TKI) targeting BCR-ABL1, revolutionized CML therapy (*2*). TKI treatment can promote long-term remission in CML patients; however, disease-initiating LSCs are resistant to TKIs despite BCR-ABL1 inhibition (*3*, *4*). Therefore, definitive cure of this disease is not yet achievable, and most CML patients still relapse upon drug discontinuation (*5*). In addition, there is a substantial risk of TKI resistance due to mutations in BCR-ABL1 (*5*) and disease progression to blast phase (*6*). Consequently, future therapies must aim at eliminating LSCs by selectively targeting pathways that are crucial for LSC homeostasis. In CML, β-catenin, a central component of the canonical Wnt pathway, is stabilized by BCR-ABL1 (*7*) and is essential for LSC self-renewal (*8*). Therefore, β-catenin deletion or its pharmacological inhibition eradicates TKI-resistant LSCs (*9*).

CD27, a costimulatory molecule of the tumor necrosis factor (TNF) receptor superfamily, is constitutively expressed on different immune cells and on hematopoietic and CML stem cells (*10*–*12*). We recently reported that CD27 signaling in LSCs promotes CML progression in a murine model (*12*). CD27 signaling is regulated by the expression of its only ligand, CD70 (*10*). CD70 is not detectable in healthy individuals but is expressed on lymphocytes and subsets of dendritic cells upon immune activation. Therefore, activated immune cells expressing CD70 may contribute to CML progression (*13*). In addition, several solid tumors and lymphomas have been shown to express CD70, but the relevance and the physiological consequences of CD70 expression on cancer cells are controversial (*10*, *14*).

Here, we demonstrate that TKI-mediated BCR-ABL1 inhibition in leukemia cell lines and CD34$^+$ stem/progenitor cells from newly diagnosed CML patients induces expression of CD70 by down-regulating microRNA-29 (*miR-29*) expression, resulting in reduced methylation of *CD70* promoter DNA and up-regulation of the transcription factor specificity protein 1 (SP1). CD70 expression induced CD27 signaling, compensatory Wnt pathway activation, and TKI resistance. Cotreatment with TKIs and monoclonal antibodies blocking the CD70/CD27 interaction synergistically reduced leukemia cell proliferation and colony formation in vitro and effectively eradicated human CD34$^+$ CML stem/progenitor cells in murine xenografts in vivo. Similarly, in a murine CML model, combination therapy eradicated LSCs in most of the treated animals. Our data reveal a therapeutically targetable mechanism of TKI resistance in CML LSCs.

RESULTS

TKI treatment induces CD70 expression in human leukemia cells

To analyze the impact of TKI treatment on CD70 expression, we cultured the BCR-ABL1$^+$ CML cell line KBM5 in the presence of imatinib. Imatinib induced *CD70* mRNA and CD70 protein expression in a dose-dependent manner (Fig. 1, A to C). The *CD70* promoter contains binding sites for several transcription factors including SP1 and is methylation-sensitive (*15*). Imatinib treatment resulted in up-regulation of *SP1* and down-regulation of DNA methyltransferase 1 (*DNMT1*) mRNA expression (Fig. 1, D and E). Similar results were obtained with the second-generation TKI nilotinib and in different BCR-ABL1$^+$ leukemia cell lines such as K562 and SD-1 (fig. S1, A to O).

In line with these findings, fluorescence-activated cell sorting (FACS)–sorted CD34$^+$ stem/progenitor cells from newly diagnosed CML patients (see table S1 for clinical characteristics of the included patients) cultured ex vivo in the presence of imatinib exhibited

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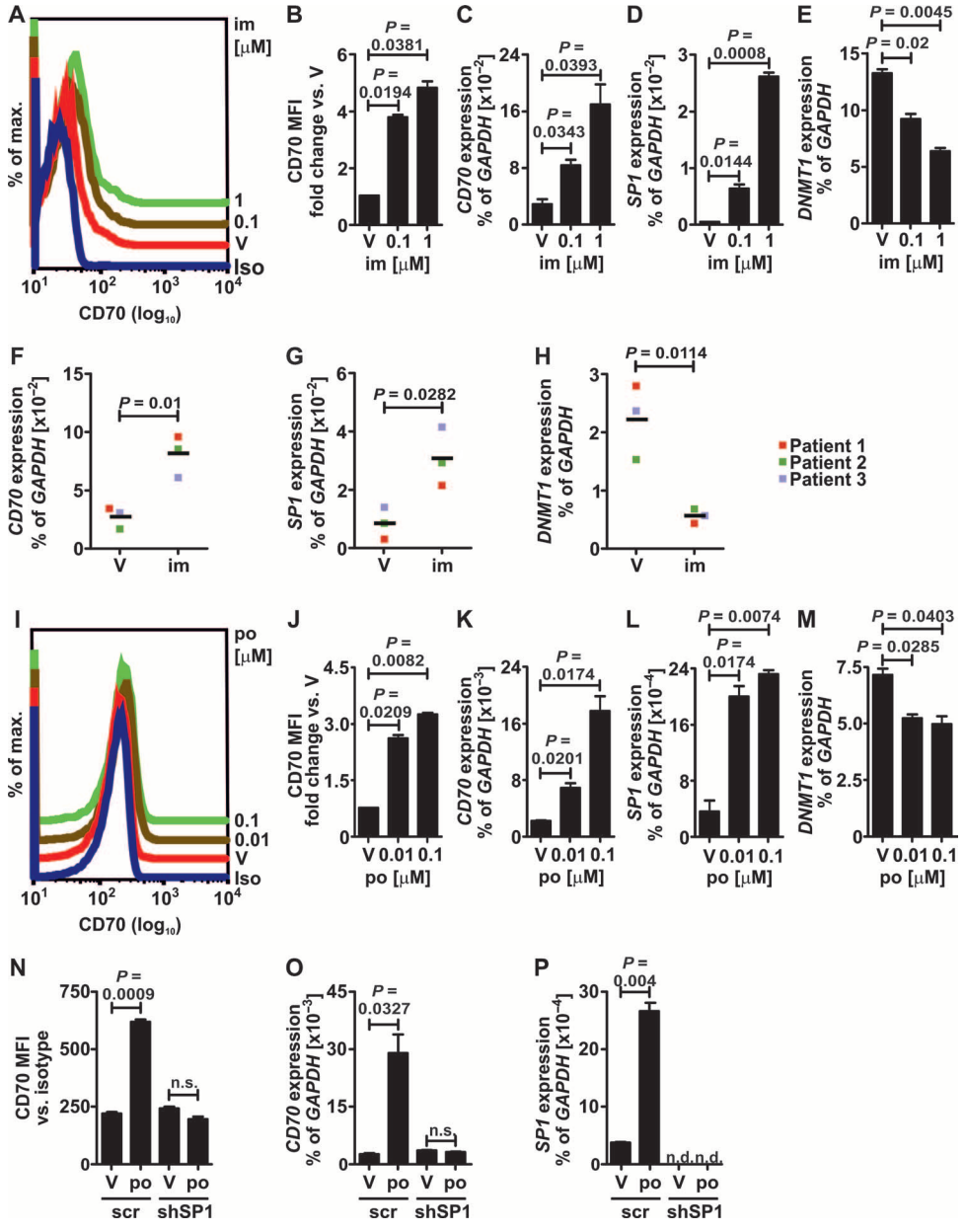

Fig. 1. TKI treatment induces CD70 expression on human CML cell lines and human CD34$^+$ CML stem/progenitor cells. (**A** to **E**) KBM5 cells (1×10^5) were cultured for 72 hours in the presence of vehicle (V; H_2O) or imatinib (im) at the indicated concentrations. Histograms (A) and mean fluorescence intensity (MFI) (B) of CD70 protein expression (FACS). *CD70* (C), *SP1* (D), and *DNMT1* (E) mRNA expression [quantitative reverse transcription polymerase chain reaction (qRT-PCR)]. (**F** to **H**) FACS-sorted CD34$^+$ stem/progenitor cells (1×10^4) from the blood of newly diagnosed CML patients were cultured in liquid culture for 7 days in the presence of vehicle or imatinib (1 μM). *CD70* (F), *SP1* (G), and *DNMT1* (H) mRNA expression (qRT-PCR). (**I** to **M**) Imatinib-resistant KBM5r cells (1×10^5) were cultured for 72 hours in the presence of vehicle or ponatinib (po) at the indicated concentrations. Histograms (I) and MFI (J) of CD70 protein expression (FACS). *CD70* (K), *SP1* (L), and *DNMT1* (M) mRNA expression (qRT-PCR). (**N** to **P**) KBM5r cells (1×10^5) transfected with shSP1 (shSP1) or the respective control scrambled RNA lentiviral particles (scr) were cultured for 72 hours in the presence of vehicle or ponatinib (po; 0.1 μM). (N) MFI of CD70 protein expression (FACS). *CD70* (O) and *SP1* (P) mRNA expression (qRT-PCR). Pooled data from two experiments run in duplicates (B to E and J to M) and data from one experiment run in duplicates are shown (N to P), respectively. Data are displayed as means ± SEM. Statistics: (B) and (J), one-sample *t* test (hypothetical value, 1); (C) to (H) and (K) to (P), Student's *t* test. n.s., not significant; n.d., not detected; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

increased *CD70* and *SP1* mRNA expression, whereas *DNMT1* was down-regulated (Fig. 1, F to H).

To determine whether the mechanism of TKI-mediated CD70 induction is dependent on BCR-ABL1 inhibition or an off-target effect, we made use of the imatinib-resistant KBM5 cell line harboring the BCR-ABL1^{T315I} mutation (referred to as KBM5r). BCR-ABL1^{T315I} represents ~20% of all clinically observed BCR-ABL1 mutations and confers resistance to three TKIs currently used in clinics (imatinib, nilotinib, and dasatinib) (*16*). At the therapeutic concentration of 1 μM, which is associated with an optimal response in CML patients (*17*), imatinib treatment did not increase the expression of CD70 protein and *CD70* or *SP1* mRNAs nor decrease the expression of *DNMT1* mRNA in KBM5r cells (fig. S2, A to E). Therefore, we cultured KBM5r cells in the presence of the pan-BCR-ABL1 inhibitor ponatinib, which was developed to overcome BCR-ABL1^{T315I} mutation–mediated resistance (*18*) and has proven effective in clinical trials (*19*). Ponatinib treatment at the therapeutic concentration of 0.1 μM (*19*) induced CD70 protein and mRNA expression, up-regulated *SP1*, and down-regulated *DNMT1* mRNA in KBM5r cells (Fig. 1, I to M). CD70 protein expression was already up-regulated after 16 hours of treatment (fig. S3A), at a time point where ponatinib did not induce substantial cell death (fig. S3B). This indicates that the observed increase in CD70 expression after TKI treatment is caused by an up-regulation of the protein rather than a selection of CD70-expressing cells.

To functionally prove that the expression of CD70 after TKI treatment is mediated by SP1, we stably knocked down SP1 in KBM5r cells using short hairpin SP1 (shSP1) lentiviral particles. SP1 knockdown KBM5r cells did not up-regulate CD70 in the presence of ponatinib compared to scrambled control RNA–transfected cells (Fig. 1, N to P).

To investigate whether the Wnt pathway represents a link between TKI-mediated BCR-ABL1 inhibition and the regulation of *CD70*, *SP1*, and *DNMT1*, we cultured TKI-treated KBM5r cells in the presence of lithium chloride (*20*) or the specific Wnt activator R-Spondin 1 (*21*). Reactivation of Wnt signaling by lithium or

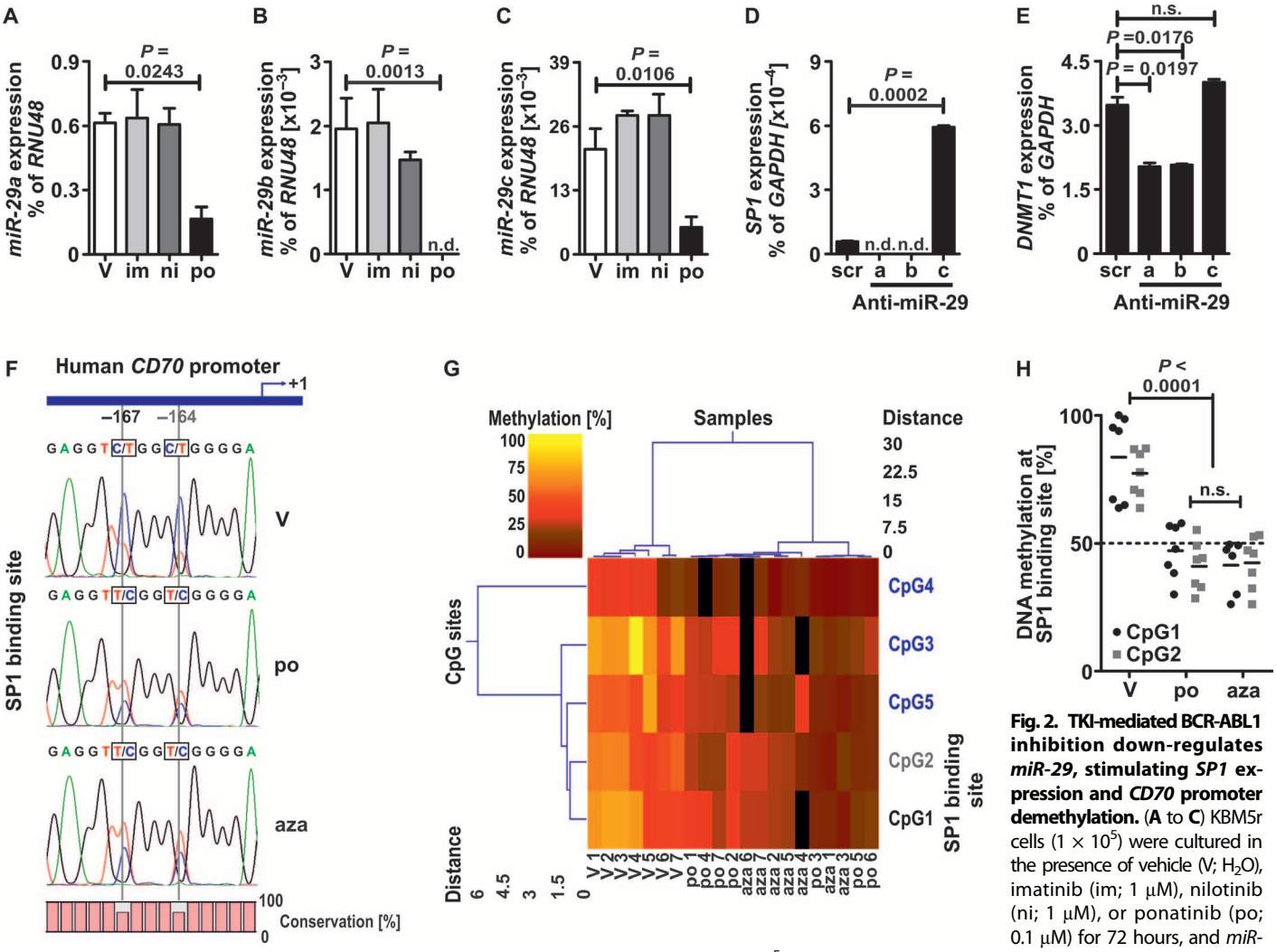

Fig. 2. TKI-mediated BCR-ABL1 inhibition down-regulates *miR-29*, stimulating *SP1* expression and *CD70* promoter demethylation. (**A** to **C**) KBM5r cells (1×10^5) were cultured in the presence of vehicle (V; H_2O), imatinib (im; 1 μM), nilotinib (ni; 1 μM), or ponatinib (po; 0.1 μM) for 72 hours, and *miR-29a* (A), *miR-29b* (B), and *miR-29c* (C) were quantified (qRT-PCR). (**D** and **E**) KBM5r cells (1×10^5) were transfected with anti–miR-29a, anti–miR-29b, anti–miR-29c, or scrambled control (scr) oligonucleotides for 48 hours, and *SP1* (D) and *DNMT1* (E) mRNA expression were analyzed (qRT-PCR). (**F**) Methylation status of the *CD70* promoter at the SP1 transcription factor binding site of KBM5r cells upon treatment with vehicle, ponatinib (0.1 μM), or azacytidine (aza) (1 μM) as determined by bisulfite sequencing. (**G**) Heat map of relative quantification of methylated cytosines at five critical CpG sites in the *CD70* promoter of KBM5r cells. (**H**) Semiquantitative analysis of DNA methylation for CpG1 and CpG2 at the SP1 binding site. (A to E) Data from one experiment each, run in duplicates, are shown. (F to H) Pooled data from three independent experiments are shown. Data are displayed as means ± SEM. Statistics: Student's *t* test. n.d., not detected.

R-Spondin 1 blocked the changes observed after TKI treatment (fig. S4, A to F).

SP1 and *DNMTs* are regulated by *miR-29* (*22*, *23*). In addition, *miR-29* has been shown to modulate Wnt signaling in a positive feedback loop (*23*, *24*). Therefore, we analyzed *miR-29* expression in KBM5r cells treated with imatinib, nilotinib, and ponatinib. Inhibition of BCR-ABL1^{T315I} by ponatinib resulted in down-regulation of all three *miR-29s*, whereas imatinib and nilotinib did not affect *miR-29s* (Fig. 2, A to C). Individual silencing of the three *miR-29* family members with anti-miRs revealed that *SP1* expression was increased after *miR-29c* silencing, whereas *DNMT1* expression was suppressed after silencing of *miR-29a* and *miR-29b* (Fig. 2, D and E).

The down-regulation of *DNMT1* by TKI treatment prompted us to investigate the DNA methylation status of the *CD70* promoter. Bisulfite sequencing revealed a strongly methylated *CD70* promoter in vehicle-treated KBM5r cells that hardly expressed any detectable CD70 protein or mRNA (Figs. 1, I to K, and 2F). Consistent with the up-regulation of CD70 mRNA and protein expression, ponatinib treatment markedly reduced *CD70* promoter DNA methylation in KBM5r cells, particularly at the SP1 binding site (Fig. 2, F to H). Control KBM5r cells cultured in the presence of the DNMT inhibitor azacytidine, a demethylating agent (*25*), showed similar reductions in *CD70* promoter methylation (Fig. 2, F to H). In summary, these data indicate that TKI-mediated blocking of BCR-ABL1, subsequent Wnt pathway inhibition, and suppression of *miR-29* induce CD70 expression by reducing its promoter methylation and up-regulating *SP1*.

Combined CD70/CD27 and BCR-ABL1 inhibition synergistically reduces Wnt signaling and eradicates leukemia cells in vitro

SD-1 cells were cultured in the presence of either a blocking αCD27 monoclonal antibody (mAb) or imatinib alone or both in combination. As previously reported (*12*), blocking the CD70/CD27 interaction reduced SD-1 cell growth by inhibiting cell proliferation (Fig. 3, A and B). In contrast, blocking CD27 signaling alone did not affect cell viability (Fig. 3C). In line with its documented ability to induce apoptosis of BCR-ABL1$^+$ cells (*26*), imatinib treatment resulted in SD-1 cell death as analyzed by FACS (Fig. 3C). Imatinib reduced SD-1 cell growth and proliferation to a similar extent as αCD27 treatment (Fig. 3, A and B). Compared to single treatments, αCD27/imatinib cotreatment significantly ($P < 0.001$) reduced cell growth by inhibiting cell proliferation and enhancing cell death (Fig. 3, A to C). Similar results were obtained by treating SD-1 cells with αCD27 mAb and nilotinib (Fig. 3, D to F), and by treating KBM5 and KBM5r cells with αCD27 mAb and imatinib or ponatinib, respectively (fig. S5).

To investigate whether the treatment combination of αCD27 mAb and imatinib resulted in synergistic activity, we performed a drug combination study according to the Chou-Talalay method (*27*). Cotreatment showed synergistic growth inhibition at all concentrations tested (Fig. 3G, fig. S6, A and B, and table S2).

We previously demonstrated that the CD70/CD27 interaction on murine CML LSCs activates the Wnt signaling pathway (*12*). TKIs such as imatinib reduce aberrant BCR-ABL1–induced Wnt signaling in CML cells (*7*). To analyze if the synergistic effect on SD-1 cell viability and proliferation of αCD27/imatinib cotreatment was mediated via Wnt pathway inhibition, we performed a lentiviral Wnt signaling reporter assay and analyzed the expression of selected Wnt target genes. αCD27 and imatinib single treatments similarly reduced Wnt signaling and Wnt target gene transcription. αCD27/imatinib cotreatment inhibited Wnt pathway activation significantly ($P < 0.001$) stronger than each of the single compounds alone (Fig. 3, H and I). In contrast, Notch, Hedgehog, and MAP (mitogen-activated protein) kinase pathways were unaffected or only minimally affected by the αCD27/imatinib cotreatment (fig. S6C).

CD70/CD27 and BCR-ABL1 co-inhibition promotes cell death of human CML stem/progenitor cells

Next, we investigated the functional role of increased CD27 signaling in response to imatinib-induced CD70 up-regulation in magnetic cell sorter (MACS)–purified human CD34$^+$ CML stem/progenitor cells. CD27 and CD70 expression were determined by FACS (Fig. 4, A to D). To

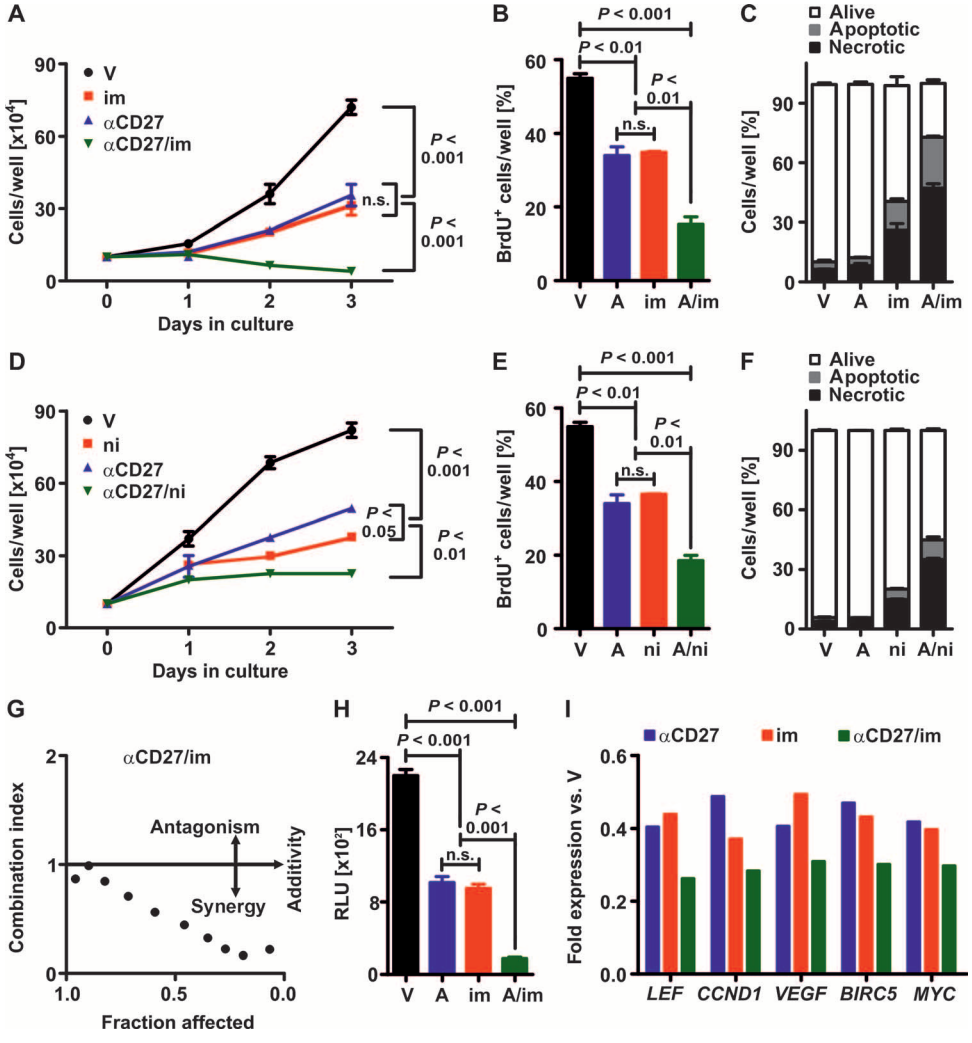

Fig. 3. BCR-ABL1 and CD70/CD27 co-inhibition synergistically eradicates SD-1 leukemia cells. (**A** to **F**) SD-1 cells (1×10^5) were cultured for 72 hours in the presence of either vehicle [V; H_2O + immunoglobulin G (IgG)], αCD27 (10 μg/ml) blocking mAb (A; H_2O + αCD27; clone 15H6), 1 μM imatinib (im; im + IgG), or 1 μM nilotinib (ni; ni + IgG) alone or both αCD27 and TKI in combination. Cell numbers (A and D), BrdU (bromodeoxyuridine) incorporation (B and E), and cell viability (C and F) were determined by trypan blue staining and FACS. Apoptotic and necrotic cells were defined as annexin V$^+$ and annexin V$^+$7-AAD$^+$ cells, respectively. (**G**) Fraction affected–combination index plot [Chou-Talalay plot (*27*)] assessing synergism and/or antagonism in an αCD27/imatinib drug combination study. Briefly, 1×10^5 SD-1 cells were treated with vehicle, αCD27, or imatinib alone or in combination in a constant ratio (see also fig. S6 and table S2). Cell numbers per well were counted after 72 hours, and the effect of drug treatment was calculated as the ratio of surviving cells to vehicle-treated cells. Fraction affected values of 0, 0.5, and 1 correspond to 0, 50, and 100% surviving cells. A combination index of <1, 1, and >1 represents synergism, additivity, and antagonism, respectively. (**H** and **I**) SD-1 cells (1×10^5) were cultured in the presence of the compounds as described for (A) to (F), and activation of the Wnt pathway was assessed using a TCF/LEF luciferase reporter assay after 24 hours (H) and by analysis for the expression of selected Wnt target genes after 72 hours (I) (qRT-PCR). One representative experiment of two run in duplicates is shown for each panel. RLU, relative luminescence units. Data are displayed as means ± SEM. Statistics: (A) and (D), two-way analysis of variance (ANOVA); (B), (E), and (H), one-way ANOVA.

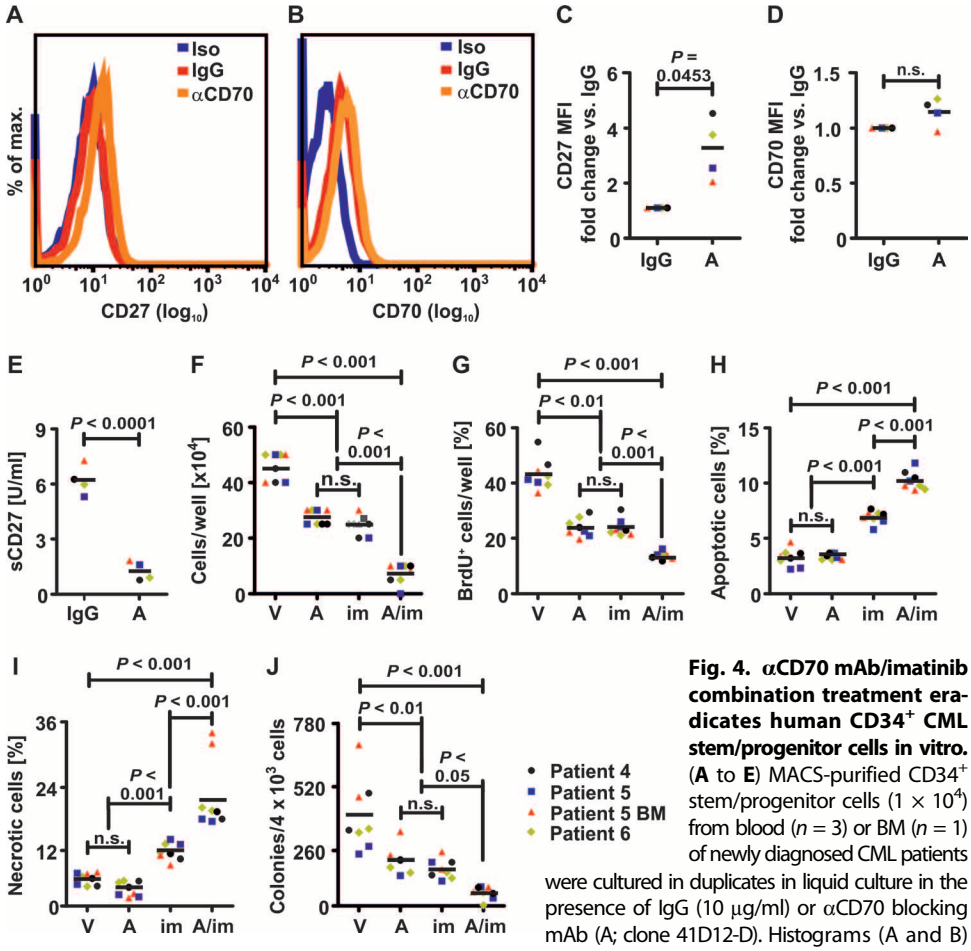

Fig. 4. αCD70 mAb/imatinib combination treatment eradicates human CD34$^+$ CML stem/progenitor cells in vitro. (**A** to **E**) MACS-purified CD34$^+$ stem/progenitor cells (1×10^4) from blood ($n = 3$) or BM ($n = 1$) of newly diagnosed CML patients were cultured in duplicates in liquid culture in the presence of IgG (10 μg/ml) or αCD70 blocking mAb (A; clone 41D12-D). Histograms (A and B) and MFI (C and D) of CD27 and CD70 expression on CD34$^+$ CML stem/progenitor cells. (E) sCD27 concentrations in supernatants after 7 days of culture. (**F** to **J**) FACS-sorted CD34$^+$ cells (1×10^4) were cultured in duplicates in liquid culture in the presence of vehicle (H_2O + IgG), αCD70 (10 μg/ml) (H_2O + αCD70), 1 μM imatinib (im; im + IgG), or both in combination (A/im; αCD70 + imatinib). Cell numbers (F), BrdU incorporation (G), and cell viability (H and I) were determined by trypan blue staining and FACS after 7 days. Apoptotic and necrotic cells were defined as annexin V$^+$ and annexin V$^+$7-AAD$^+$ cells, respectively. (J) Duplicates of 4×10^3 FACS-sorted CD34$^+$ CML stem/progenitor cells were cultured overnight in 96-well V-bottom plates in the presence of the compounds as described for (B) to (K) and were then transferred into methylcellulose containing the respective drugs. Colony formation was determined after 14 days by inverted light microscopy. Statistics: (C) and (D), one-sample *t* test (hypothetical value, 1); (E), Student's *t* test; (F) to (J), one-way ANOVA.

determine the extent of the CD70/CD27 interaction (CD27 signaling) in the different patient samples, we measured soluble CD27 (sCD27) in the cell supernatants. Ligation of CD27 by CD70 results in shedding of CD27 from the cellular membrane and release of sCD27 (*10*). Treatment of CD34$^+$ CML stem/progenitor cells with a blocking human αCD70 mAb (clone 41D12-D) resulted in up-regulation of membrane-bound CD27 (Fig. 4, A and C) and a lower concentration of sCD27 (Fig. 4E). The expression of CD70 was not altered after αCD70 mAb treatment (Fig. 4, B and D). sCD27 concentrations were very comparable in all supernatants of untreated patient samples and were similarly reduced after αCD70 treatment. This indicates that the extent of CD27 ligation was quite similar between patient samples.

Single treatments with either αCD70 mAb or imatinib inhibited growth and proliferation of FACS-sorted CD34$^+$ CML stem/progenitor cells (Fig. 4, F and G). The human αCD70 mAb (clone 41D12-D) was specifically designed to block the CD70/CD27 interaction without inducing effector functions such as antibody-dependent cell- or complement-mediated cytotoxicity and antibody-dependent cell-mediated phagocytosis (*28*). In line with our results using the blocking αCD27 mAb and SD-1 cells (Fig. 3, A to F), single αCD70 treatment inhibited cell proliferation without affecting cell viability, whereas single imatinib treatment reduced cell proliferation and induced cell death (Fig. 4, G to I). αCD70/imatinib cotreatment potently reduced CD34$^+$ CML stem/progenitor cells in liquid cultures by inhibiting cell proliferation and increasing cell death (Fig. 4, G to I). In addition, colony formation in semisolid cultures was significantly ($P < 0.05$) impaired by cotreatment compared to single treatments (Fig. 4J). Control CD34$^+$ hematopoietic stem/progenitor cells from patients who underwent bone marrow (BM) aspirate for reasons other than leukemia ("healthy donors") were only marginally affected by αCD70 or imatinib treatment in vitro (fig. S7, A to E).

αCD70/imatinib cotreatment eradicates human CD34$^+$ CML stem/progenitor cells in murine xenografts

To validate our findings in vivo, we used a previously described murine CML xenograft model (*29*, *30*) in nonobese diabetic (NOD)/LtSz-*scid* IL2Rγ^{null} (NSG) mice (*31*). MACS-sorted CD34$^+$ CML stem/progenitor cells (2×10^6) from the blood of patients 3, 4, and 5 carrying different BCR-ABL1 translocations (table S1) were injected intravenously into sublethally irradiated (2.75 Gy) NSG mice. After 7 days of engraftment, NSG mice were randomized to receive vehicle, imatinib, or αCD70 treatment alone or αCD70/imatinib cotreatment. Plasma concentrations of imatinib in xenografted NSG mice were close to the published therapeutic concentration of 1 μM (fig. S8) (*17*). After 2 weeks of treatment, animals were sacrificed, and the BM was analyzed for human CD45$^+$CD33$^+$ CML myeloid cells and CD45$^+$CD34$^+$ CML stem/progenitor cells (Fig. 5, A to E). At that time point, vehicle-treated NSG mice had an average of 11.8 ± 1.1% of human CD45$^+$CD33$^+$ CML myeloid cells in the BM (Fig. 5B). Single αCD70 or imatinib treatment reduced cell frequencies and absolute numbers of CD45$^+$CD33$^+$ leukemia cells and leukemia-initiating CD34$^+$ CML stem/progenitor cells (Fig. 5, C to E). αCD70/imatinib cotreatment further reduced CD45$^+$CD33$^+$ CML myeloid cells (Fig. 5, C and D) and eradicated the leukemia-initiating CD34$^+$ CML stem/progenitor cells in the BM of 9 of 12 NSG mice (Fig. 5E).

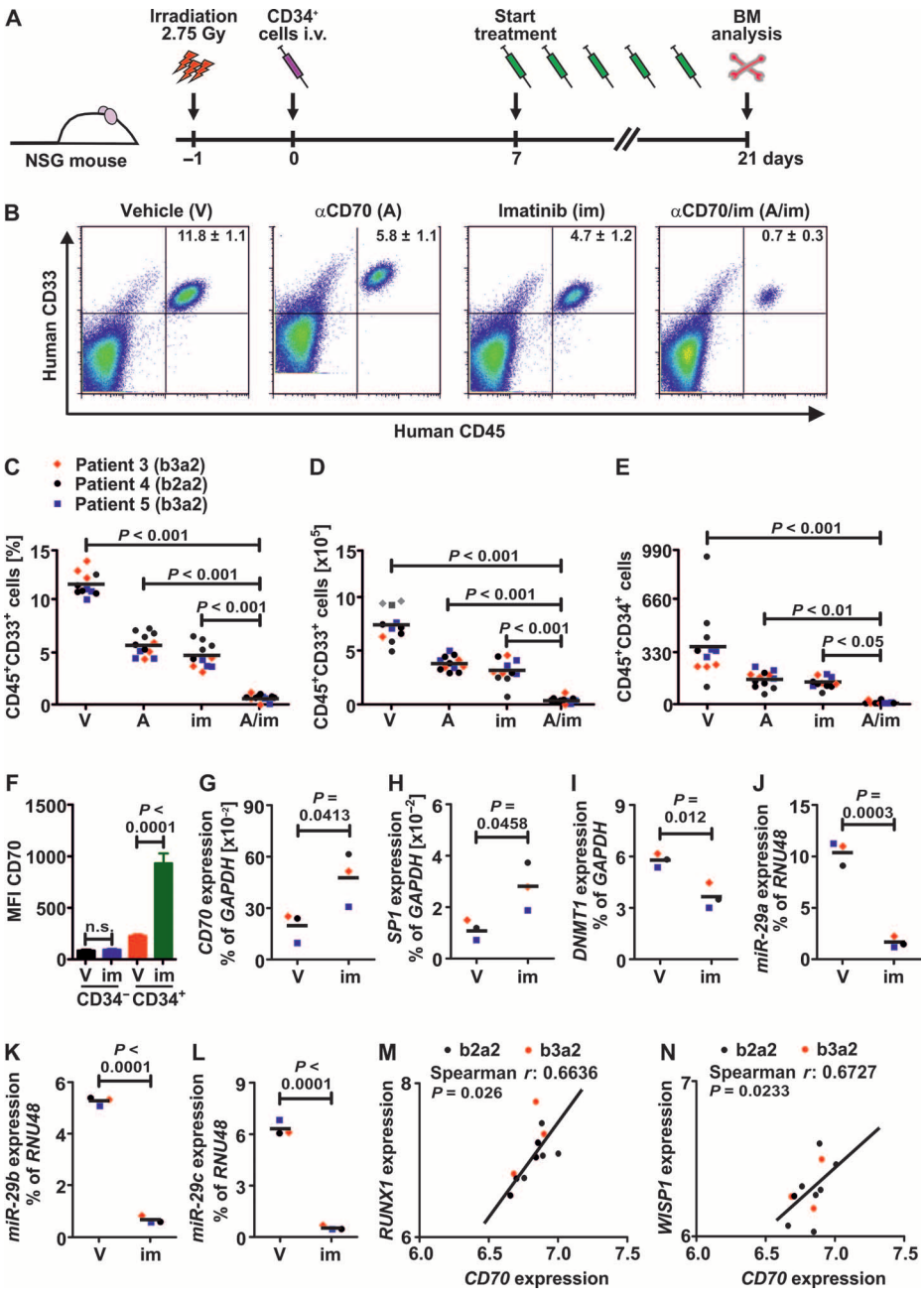

Fig. 5. αCD70 mAb/imatinib combination therapy eradicates human CD34$^+$ CML stem/progenitor cells in murine xenografts. (**A** to **E**) MACS-purified CD34$^+$ stem/progenitor cells (2×10^6) from the blood of three newly diagnosed CML patients were injected intravenously (i.v.) into irradiated (2.75 Gy) NSG mice. Starting 1 week after transplantation, imatinib (im; 50 mg/kg) was administered once daily by oral gavage. (A) αCD70 (10 mg/kg) was administered intraperitoneally every third day. Sterile H_2O and a control mAb specific for the F protein of respiratory syncytial virus (palivizumab) were used as mock treatment (V). (B) After 2 weeks of treatment, mice were euthanized, and BM was analyzed for human cells by FACS. Percentages (C) and absolute numbers (D) of human CD45$^+$CD33$^+$ CML myeloid cells and absolute numbers of human CD45$^+$CD34$^+$ CML stem/progenitor cells in the BM (E). (**F**) MFI of CD70 expression on CD45$^+$CD34$^-$ CML cells and CD45$^+$CD34$^+$ CML stem/progenitor cells in xenografted NSG mice (n = 3 per xenograft and per treatment). (**G** to **L**) *CD70* (G), *SP1* (H), *DNMT1* mRNA (I), and *miR-29* expression (J to L) (qRT-PCR) in CD34$^+$ cells. (**M** and **N**) Correlation of *CD70* expression with the expression of selected Wnt target genes *RUNX1* (M) and *WISP1* (N) in CD34$^+$ stem/progenitor cells from newly diagnosed chronic phase CML patients. Expression data are derived from a public repository for microarray data and are available under accession number E-MEXP-480 (www.ebi.ac.uk/arrayexpress). Data are displayed as means ± SEM. Statistics: (C) to (E), one-way ANOVA; (F) to (L), Student's t test; (M) and (N), Spearman correlation.

CD34$^+$ CML stem/progenitor cells isolated ex vivo from xenografted vehicle-treated NSG mice expressed CD70 (Fig. 5F and fig. S9A) but only low levels of CD27 (fig. S9, B and C). CD70 and CD27 were not expressed on more differentiated CD34$^-$ CML cells (Fig. 5F and fig. S9C). Imatinib treatment of xenografted NSG mice specifically increased CD70 expression on CD34$^+$ CML stem/progenitor cells but not on CD34$^-$ CML cells (Fig. 5, F and G). In contrast, the expression of CD27 remained unchanged after TKI treatment (fig. S9C). In addition, imatinib treatment induced an up-regulation of *SP1* and decreased the expression of *DNMT1* and *miR-29a*, *miR-29b*, and *miR-29c* in CD34$^+$ CML stem/progenitor cells in vivo (Fig. 5, H to L).

To analyze if CD70 expression correlates with Wnt pathway activation in CML patients harboring the b3a2 or the b2a2 BCR-ABL1 translocation, we made use of expression data derived from a public repository for microarray data (accession number E-MEXP-480; www.ebi.ac.uk/arrayexpress). Independent of the BCR-ABL1 translocation, CD70 expression positively correlated with the expression of Wnt target genes (Fig. 5, M and N).

TKI treatment induces CD70 expression in murine CML LSCs

Because human CD34$^+$ CML stem/progenitor cells do not engraft long term in NSG mice (*29*), we also tested our cotreatment strategy in a well-established syngeneic CML model (*12*). BM from donor BL/6 mice was transduced with BCR-ABL1–green fluorescent protein (GFP) and injected into sublethally irradiated (4.5 Gy) recipients. First, we intended to study the effects of imatinib single treatment on the expression of CD70 protein and *Cd70*, *Sp1*, and *Dnmt1* mRNA in murine LSCs in vivo. Similar to murine hematopoietic stem cells (HSCs) (*32*), murine LSCs reside in a BM cell population characterized by the lack of lineage markers and by the expression of stem cell antigen-1 (Sca-1) and c-kit [lin$^-$Sca-1$^+$c-kit^{hi} cells (LSKs); fig. S10] (*33*). CML-bearing mice were treated

with vehicle or imatinib, and CD70 expression in BCR-ABL1-GFP$^+$ LSKs was analyzed. Consistent with our data obtained for KBM5 cells and human CD34$^+$ CML stem/progenitor cells in vitro, BCR-ABL1-GFP$^+$ LSKs from imatinib-treated CML mice expressed higher amounts of CD70 protein and mRNA as compared to BCR-ABL1-GFP$^+$ LSKs from vehicle-treated mice (Fig. 6, A to C, and fig. S10). Moreover, imatinib treatment increased *Sp1* and decreased *Dnmt1* mRNA in BCR-ABL1-GFP$^+$ LSKs (Fig. 6, D and E). CD70 up-regulation upon imatinib treatment was specific for BCR-ABL1-GFP$^+$ LSKs (figs. S10 and S11A), and imatinib did not alter CD70 expression on BCR-ABL1-GFP$^+$Sca-1$^-$ leukemia progenitors (fig. S11A). Furthermore, CD70, *Sp1*, and *Dnmt1* expression did not change in endogenous nonmalignant GFP$^-$ LSKs upon imatinib treatment (Fig. 6A and figs. S10 and S11, B to E).

As previously demonstrated, the CD70/CD27 interaction on murine CML LSCs activates the Wnt signaling pathway via TNF receptor–associated factor 2 (TRAF2) and the TRAF2- and NCK-interacting protein kinase (TNIK) (*12*). Although imatinib treatment did not affect CD27 expression on BCR-ABL1-GFP$^+$ LSKs, *Traf2* and *Tnik* were up-regulated at the mRNA level, whereas Wnt target genes such as *Runx1* and *Myc* were down-regulated (fig. S11, F to J). These results are in line with and further support the hypothesis that TKIs specifically alter the expression of CD70, SP1, and DNMT1 via BCR-ABL1 inhibition and increase the expression of CD27 downstream signaling molecules such as TRAF2 and TNIK.

αCD70/imatinib combination therapy eliminates murine CML LSCs in vivo

We also analyzed the effect of combining TKI treatment with CD70/CD27 inhibition on the survival of CML mice. Fifteen days after

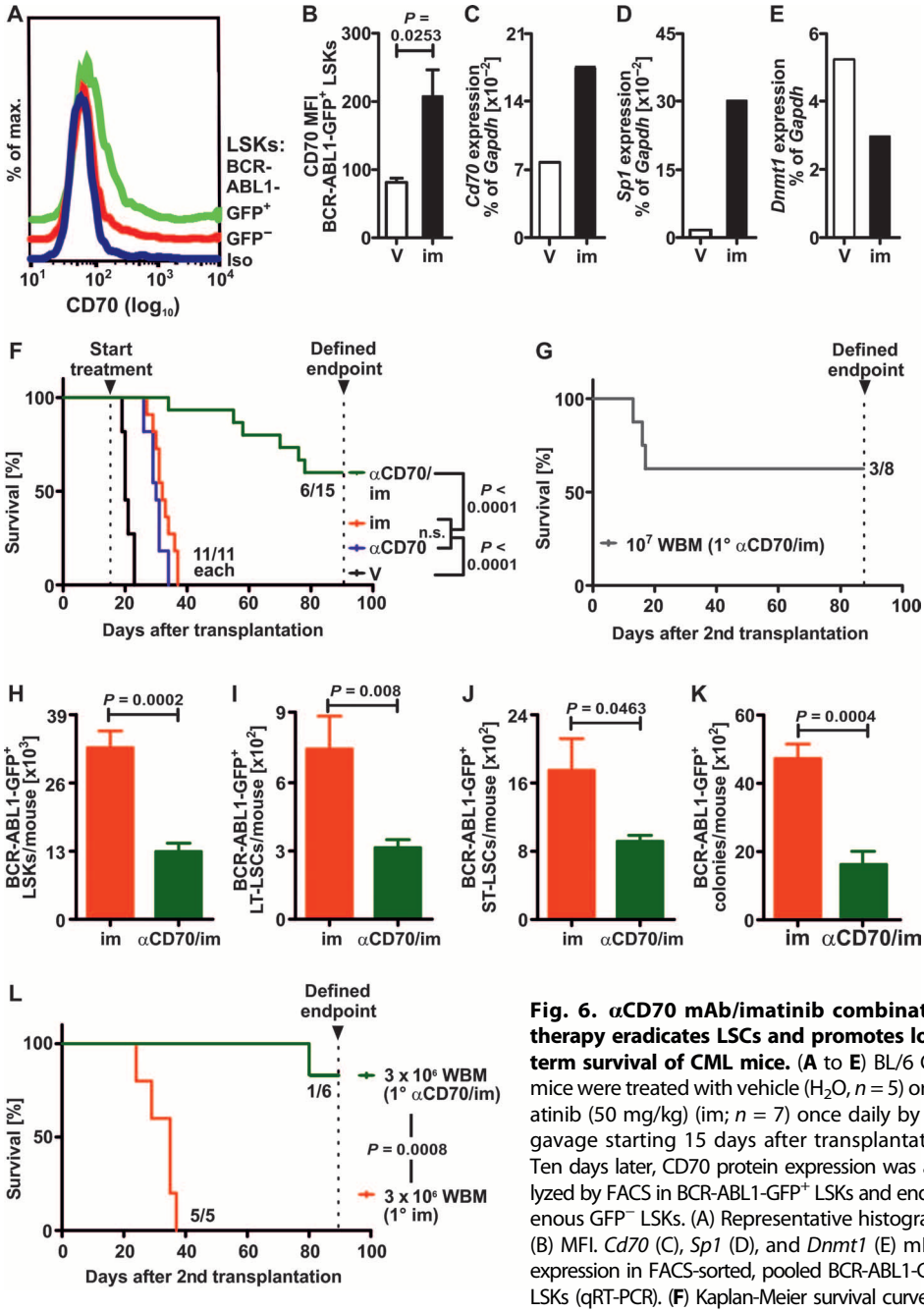

Fig. 6. αCD70 mAb/imatinib combination therapy eradicates LSCs and promotes long-term survival of CML mice. (**A** to **E**) BL/6 CML mice were treated with vehicle (H_2O, $n = 5$) or imatinib (50 mg/kg) (im; $n = 7$) once daily by oral gavage starting 15 days after transplantation. Ten days later, CD70 protein expression was analyzed by FACS in BCR-ABL1-GFP$^+$ LSKs and endogenous GFP$^-$ LSKs. (A) Representative histograms. (B) MFI. *Cd70* (C), *Sp1* (D), and *Dnmt1* (E) mRNA expression in FACS-sorted, pooled BCR-ABL1-GFP$^+$ LSKs (qRT-PCR). (**F**) Kaplan-Meier survival curves of primary BL/6 CML mice. Starting 15 days after transplantation, imatinib (50 mg/kg) was administered once daily by oral gavage. αCD70 (300 μg) blocking mAb (clone FR70) was administered intraperitoneally every third day. Sterile H_2O and IgG from rat serum were used as controls. Pooled data from two independent experiments with $n =$ 11 to 15 mice per group are shown. (**G**) Survival of lethally irradiated (2 × 6.5 Gy) secondary recipients ($n = 8$) that received 1×10^7 whole-BM (WBM) cells from αCD70/im-treated primary CML mice ($n = 8$) that were alive 90 days after primary transplantation. Data from one primary transplantation experiment with $n = 8$ surviving animals are shown. (**H** to **L**) Primary BL/6 CML mice were treated with imatinib alone ($n = 5$) or with the combination therapy ($n = 6$) as described in (F), starting 15 days after transplantation. Ten days later, numbers of BCR-ABL1-GFP$^+$ LSKs (H), BCR-ABL1-GFP$^+$ LT-LSCs (LSK CD135$^-$CD48$^-$CD150$^+$) (I), and BCR-ABL1-GFP$^+$ ST-LSCs (LSK CD135$^-$CD48$^-$CD150$^-$) (J) were determined in the BM (FACS). (K) Equal numbers of total lin$^-$ cells were plated in methylcellulose, and BCR-ABL1-GFP$^+$ colonies were enumerated 7 days later by inverted fluorescence microscopy. (L) WBM cells (3×10^6) were transplanted into sublethally irradiated (4.5 Gy) recipient mice, and survival was monitored. (F, G, and L) Numbers of mice that succumbed to CML of total transplanted mice are indicated. Data are displayed as means ± SEM. Statistics: (B) and (H) to (K), Student's *t* test; (F) and (L), log-rank test.

transplantation, mice harboring comparable leukemia loads (49 ± 5 BCR-ABL1-GFP$^+$Gr-1$^+$ granulocytes per microliter of blood) were randomized to monotherapy with vehicle, imatinib, or a blocking αCD70 mAb (clone FR70) (*34*, *35*) or αCD70/imatinib combination therapy, and disease development and survival were monitored. Monotherapy delayed leukemia development and prolonged survival compared to the vehicle group; nevertheless, all animals eventually succumbed to CML. αCD70/imatinib combination therapy significantly ($P < 0.001$) improved survival of CML mice compared to monotherapy, and 60% (9 of 15) of the animals receiving the combination therapy were alive 90 days after transplantation (Fig. 6F). This suggested that CML stem/progenitor cells were completely eradicated or at least effectively controlled long term in these mice.

To investigate this issue, surviving mice were sacrificed, and spleen weights and BM BCR-ABL1-GFP$^+$lin$^-$ cell frequencies were determined. In this experiment, one of eight surviving mice displayed splenomegaly; this and two other mice harbored detectable levels of BCR-ABL1-GFP$^+$lin$^-$ cells in the BM, indicating the presence of CML (fig. S12, A and B). Secondary transplantation of 1×10^7 WBM cells from these three mice induced fatal CML in lethally irradiated (2 × 6.5 Gy) BL/6 recipients (Fig. 6G). In contrast, secondary recipients receiving WBM cells from the other five donors survived up to 90 days without signs of leukemia (Fig. 6G). This indicates that αCD70/imatinib cotreatment targeted and eliminated the disease-initiating LSCs in five of eight of the primary CML animals.

The BCR-ABL1-GFP$^+$ LSK cell population that contains the LSCs is heterogeneous and hierarchically organized, and can be further subdivided into long-term LSCs (LT-LSCs), short-term LSCs (ST-LSCs), and leukemia multipotent progenitors (MPPs) using the markers CD150, CD135, and CD48 (*30*, *36*). To analyze the impact of combination therapy on these LSC subpopulations in more detail, especially on the disease-initiating LT-LSCs (*36*), CML mice harboring comparable leukemia loads (163 ± 20 BCR/ABL1-GFP$^+$Gr-1$^+$ granulocytes per microliter of blood) 15 days after transplantation received either imatinib alone or αCD70/imatinib combination therapy. Ten days later, mice were sacrificed, and BM and spleen were analyzed. Compared to imatinib monotherapy, αCD70/imatinib combination therapy resulted in lower spleen weights and a lower leukemia load as indicated by lower numbers of BCR-ABL1-GFP$^+$Gr-1$^+$ granulocytes in the blood as well as lower numbers of BCR-ABL1-GFP$^+$lin$^-$ leukemia cells and BCR-ABL1-GFP$^+$lin$^-$c-kit^{hi} leukemia progenitors in the BM (fig. S12, C to F). αCD70/imatinib combination therapy more efficiently eliminated the BCR-ABL1-GFP$^+$ LSK cell population, including the disease-initiating LT-LSCs, than did imatinib monotherapy (Fig. 6, H to J, and fig. S12, G and H).

In addition, lin$^-$ BM cells from αCD70/imatinib-cotreated CML animals formed significantly ($P = 0.0004$) fewer BCR-ABL1-GFP$^+$ colonies than lin$^-$ cells from imatinib-treated mice (Fig. 6K). To further confirm that the findings from FACS analysis and colony assays in vitro actually account for reduced numbers of LSCs in vivo, 3×10^6 WBM cells from imatinib- or αCD70/imatinib-treated CML mice were transplanted into sublethally irradiated (4.5 Gy) secondary recipients. Recipients that received WBM cells from imatinib-treated leukemia mice all succumbed to CML. In contrast, five of six mice receiving WBM cells from mice that had been treated with the αCD70 mAb/imatinib combination therapy completely eliminated the disease and were alive 90 days after secondary transplantation without signs of leukemia (Fig. 6L). In summary, these data provide evidence that combination therapy using TKI and CD70/CD27 inhibition selectively targets CML LSCs, particularly the disease-initiating LT-LSCs in vivo.

DISCUSSION

In myeloid leukemias, intrinsic and extrinsic factors mediate drug resistance in LSCs and constitute important barriers to current treatment strategies (*37*). Intrinsic drug resistance mechanisms, such as quiescence, the expression of adenosine 5′-triphosphate–binding cassette (ABC) transporters, and preexisting or acquired BCR-ABL1 mutations, protect LSCs from both DNA-damaging agents and TKIs (*1*, *37*, *38*). Several approaches were proposed to overcome these intrinsic resistance mechanisms, including two-step therapy regimens using cell cycle–activating drugs followed by TKIs or chemotherapy (*39*) and the specific targeting of ABC transporters (*40*). Furthermore, next-generation TKIs with improved efficacy against mutated BCR-ABL1 were successfully developed and introduced into clinical practice (*18*). However, recent studies demonstrated that LSC survival and persistence are independent of BCR-ABL1 (*3*, *4*), and therefore, even next-generation TKIs will most likely be ineffective against LSCs when used as monotherapy.

In addition, accumulating evidence indicates that extrinsic factors from the BM microenvironment play an essential role in regulating LSC survival, self-renewal, and drug resistance (*37*, *41*). Similarly to HSCs, LSCs reside in and depend on BM niches consisting of cell types such as osteoblasts, mesenchymal stromal cells (MSCs), and endothelial cells (*13*, *42*). These niche cells provide soluble and cell contact–dependent signals, including cytokines, chemokines, Wnt, Notch, and Hedgehog ligands, as well as integrins and cadherins that maintain LSC homeostasis (*42*). MSCs have been shown to support LSC adhesion and promote TKI resistance via N-cadherin and β-catenin (*43*). Moreover, in leukemia, BM-infiltrating immune cells may contribute to the LSC niche, and activated T cells in particular are known to interact with and paradoxically support LSCs (*12*, *13*, *44*).

The canonical Wnt pathway is essential for the maintenance of stem cell characteristics of LSCs such as quiescence, self-renewal, and the regulation of cell fate (*8*, *9*). In CML, BCR-ABL1 induces stabilization of β-catenin and thereby contributes to Wnt pathway activation (*7*). However, although BCR-ABL1–specific TKIs inhibit β-catenin stability and thereby Wnt signaling, it is well documented that LSCs are resistant to TKI treatment (*3*, *4*). Here, we document that in response to TKI treatment and Wnt pathway inhibition, CML stem cells up-regulate the expression of CD70. The CD70/CD27 interaction leads to compensatory Wnt pathway activation and thereby confers resistance of LSCs to TKI treatment. Under physiological conditions, CD70 is only expressed by activated lymphocytes and subsets of dendritic cells (*10*). In CML, activated CD70-expressing immune cells induce Wnt signaling in CD27-expressing LSCs, promoting disease progression (*12*). However, CD70 is also expressed after malignant transformation and has been detected on lymphomas and several solid tumors (*10*). CD70 was expressed at very low levels on malignant CML cells and was up-regulated on LSCs upon treatment with TKIs. It has been reported previously that CD70 expression is similarly induced upon irradiation on human glioblastoma and prostate carcinoma cells (*45*, *46*). In response to TKI treatment, LSCs express both CD27 and CD70. Therefore, CD27 signaling could be induced in a cell-autonomous and/or paracrine manner. However, in vivo, LSCs are thought to

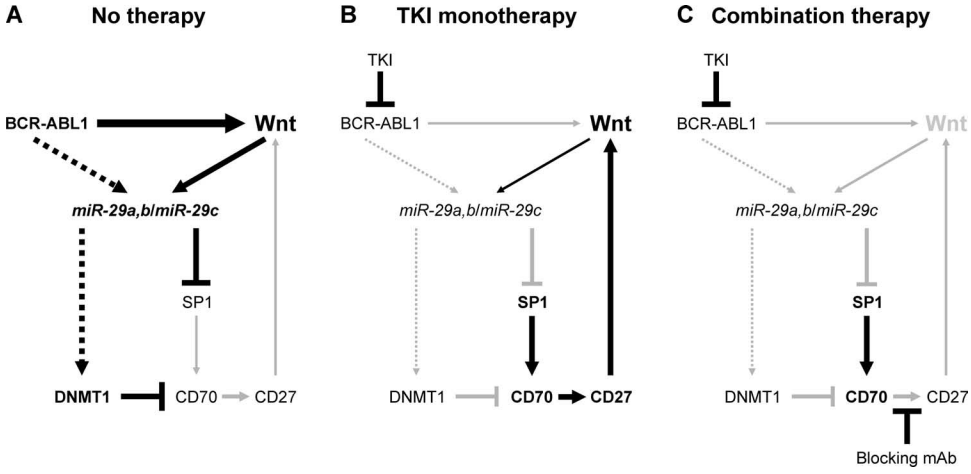

Fig. 7. Combination therapy affects the Wnt pathway. The Wnt pathway is crucial for LSC survival and maintenance (*8*, *9*). During homeostasis, the absence of Wnt ligand/frizzled receptor signaling stimulates β-catenin degradation via the proteasome (*53*). (**A**) In CML, the Wnt pathway is constantly active even in the absence of Wnt ligands because of BCR-ABL1–mediated β-catenin stabilization/activation (*7*). (**B**) TKI treatment reduces BCR-ABL1–mediated Wnt pathway activation but simultaneously represses *miR-29s*. Down-regulation of *miR-29c* results in SP1 up-regulation, whereas *miR-29a,b* down-regulation is associated with a decrease in DNMT1 expression, causing *CD70* promoter DNA demethylation. Together, these effects increase the expression of CD70, promoting CD27 signaling and compensatory Wnt pathway activation. (**C**) Blocking of CD70/CD27 signaling by mAb in combination with TKI treatment synergistically inhibits the Wnt pathway and results in LSC eradication. Solid lines, direct effects; dashed lines, indirect effects; black lines, strong effects; gray lines, weak effects.

be a rare cell population present as single cells in the BM in a specialized niche microenvironment in analogy to HSCs (*13*, *42*). Therefore, it is most likely that CD27 signaling triggered by CD70 is induced in a cell-autonomous manner.

We found that BCR-ABL1 inhibition reduced *miR-29* expression. Our data with lithium- or R-Spondin 1–induced activation of the Wnt pathway agree with earlier results in human MSCs, indicating that Wnt signaling directly increases *miR-29* expression (*24*). Down-regulation of *miR-29c* increased the expression of its direct target SP1, a transcription factor with a binding site in the *CD70* promoter (*22*), and *SP1* knockdown prevented CD70 up-regulation in response to TKI. In addition, *miR-29a/b* down-regulation was associated with a reduced expression of *DNMT1*, resulting in *CD70* promoter demethylation. These combined effects promoted CD70 up-regulation on LSCs, increased CD70/CD27 signaling, and subsequently restored Wnt pathway activity (Fig. 7). We hypothesize that during BCR-ABL1 inhibition, constant CD70 expression on LSCs is needed to sustain compensatory Wnt signaling. Therefore, blocking CD70/CD27 signaling in combination with TKIs synergistically inhibited the Wnt pathway and eradicated leukemia cells in vitro. Additionally, combination therapy effectively eliminated the disease-initiating LSCs in murine xenografts and in the murine CML model in vivo. However, because combination therapy resulted in the elimination of LSCs, we could not investigate whether CD70-triggered Wnt signaling regulates CD70 expression. In summary, our study indicates that blocking CD70/CD27 signaling in combination with TKI treatment overcomes compensatory Wnt activation and represents an attractive therapeutic strategy to specifically target CML LSCs.

MATERIALS AND METHODS

Study design

In hypothesis-driven experimental designs, we addressed the molecular mechanisms of TKI-induced CD70 expression in CML cell lines and blood or BM samples from newly diagnosed CML patients treated with TKIs in vitro. Combination therapy with TKIs and blocking mAb was applied, and its effect on Wnt pathway activation, CML cell growth, and LSC activity was assessed. These investigations were extended to a murine model of CML and to patient-derived xenografts using $CD34^+$ stem/progenitor cells from newly diagnosed CML patients to analyze the effects of combination therapy in vivo. All experiments were performed in 6- to 8-week-old mice housed in a specific pathogen–free facility in individually ventilated cages. Food was provided ad libitum. Mice were assigned randomly to the different treatment groups. Experiments were conducted and analyzed in a nonblinded fashion. Experiments were performed one to two times. Details on replicates are indicated in the figure legends.

Antibodies and reagents for treatment

Murine αCD70 (clone FR70) was from BioXCell, and control IgG from rat serum (rat IgG) was from Sigma-Aldrich. Human αCD27 (clone 1A4) and the corresponding isotype control (clone 15H6) were from Beckman Coulter. Human αCD70 (clone 41D12-D) and a corresponding control mAb specific for the F protein of respiratory syncytial virus (palivizumab) were provided by arGEN-X. Imatinib and nilotinib were provided by Novartis. Ponatinib was from Selleck Chemicals. Lithium chloride was from Sigma-Aldrich. Recombinant human R-Spondin 1 was from R&D Systems.

Patient samples

Peripheral blood samples and one BM aspirate from untreated, newly diagnosed CML patients at the Department of Hematology, Inselspital, University Hospital and University of Bern, Switzerland, were obtained after written informed consent. Patient characteristics are listed in table S1. Analysis of blood and BM samples was approved by the local ethical committee of the Canton of Bern.

In vitro experiments

Cell lines. The human leukemia cell lines SD-1, K562, KBM5, and KBM5r have been described before (*47*–*49*).

Liquid culture of primary human $CD34^+$ CML stem/progenitor cells. $CD34^+$ stem/progenitor cells of human CML patients were cultured and analyzed as previously described (*44*).

Colony assays. Colony assays with FACS-purified $CD34^+$ stem/progenitor cells from CML patients or MACS-purified BM lin^- cells from CML mice were performed as previously described (*44*).

DNA methylation analysis of the *CD70* promoter. Bisulfite conversion of isolated DNA samples from KBM5r cells was assessed using the EpiTect Bisulfite Kit (Qiagen) according to the manufacturer's protocol. The promoter region covering binding sites for important transcription factors was selectively amplified using the following primers: forward primer (−227 to −205), 5′-GTTTTAGAAGAATGAGGTGGAG-3′; reverse primer (+14 to +35), 5′-TCAACCTATCAAAAAACCAAC-3′. For the amplification of bisulfite-treated genomic DNA, the following PCR conditions were used: 1 × 95°C for 10 min; 40 × 95°C for 30 s, 58°C for 40 s, and 72°C for 1 min; and 1 × 72°C for 5 min. The PCR cocktail consisted of 3 μl of DNA (of at least 10 ng/μl DNA for a final concentration of 3 ng/μl per reaction) in a 25-μl total volume using 0.5 pM of each primer, 200 μM deoxynucleotide triphosphates, 0.2 U of Hot Start Taq DNA polymerase, 1.5 mM $MgCl_2$, and the buffer supplied with the enzyme. The amplified promoter region was gel-purified and subjected to fluorescent Sanger sequencing (*50*). The relative quantification of the methylated allele (C) versus unmethylated allele (T) was assessed by the QSV Analyser software (*51*). The ratio of methylated to unmethylated cytosine was used for the two-way hierarchical clustering analysis. The variable CpG fragments at the *CD70* promoter were clustered on the basis of pairwise Euclidean distances and linkage algorithm for all 21 samples (7 independent replicates per condition) as previously described (*50*). The procedure was performed using the double dendrogram function of the Gene Expression Statistical software (NCSS).

Animal experiments

Animal experiments were approved by the local experimental animal committee of the Canton of Bern and performed according to Swiss laws for animal protection.

Mice. C57BL/6J (BL/6) mice were from RCC Ltd. NSG mice have been described before (*31*).

Murine xenografts. MACS-purified $CD34^+$ stem/progenitor cells (2×10^6) from the blood of a newly diagnosed CML patient (patient 4) were injected intravenously into previously sublethally irradiated (2.75 Gy) NSG mice. Starting 1 week after transplantation, imatinib (50 mg/kg) was administered once daily by oral gavage. The αCD70 mAb (clone 41D12-D) (10 mg/kg) was administered intraperitoneally every third day. Sterile H_2O and palivizumab were used as control treatments. After 2 weeks of treatment, mice were euthanized, and BM from femurs and tibias was analyzed by FACS for human cell engraftment using human αCD45, αCD34, αCD33, αCD3, and αCD19 antibodies.

Murine CML model. CML was induced and monitored as described (*12*, *44*, *52*). Briefly, donor mice were treated with 5-fluorouracil (150 mg/kg) intraperitoneally. Six days later, BM was harvested and transduced twice with BCR-ABL1-GFP retrovirus by spin infection (*52*). Cells (1×10^5) were injected intravenously into the tail vein of sublethally irradiated (4.5 Gy) syngeneic recipients.

Treatment of CML mice was started 15 days after transplantation. Imatinib (50 mg/kg) was administered once daily by oral gavage. αCD70 mAb (clone FR70) (300 μg per injection) was administered intraperitoneally every third day. Sterile H_2O and rat IgG were used as control treatments. To detect residual LSC activity in surviving CML mice (90 days after primary transplantation), 1×10^7 WBM cells were injected intravenously into lethally irradiated (6.5 Gy twice with 4-hour interval) recipient mice. To compare LSC activity in mice receiving imatinib monotherapy versus αCD70/imatinib combination therapy, primary CML mice were treated for 10 days, then sacrificed, and 3×10^6 WBM cells were injected intravenously into sublethally irradiated (4.5 Gy) recipient mice.

LSC analysis. The LSC numbers in CML mice were analyzed by FACS as previously described (*30*, *44*). Briefly, LSC subpopulations in lin^-BCR-ABL1-GFP^+ BM cells were defined as follows: LT-LSCs (Sca-1^+c-$kit^{hi}CD135^-CD48^-CD150^+$), ST-LSCs (Sca-$1^+$c-$kit^{hi}CD135^-CD48^-CD150^-$), MPP1s (Sca-$1^+$c-$kit^{hi}CD135^-CD48^+CD150^+$), and MPP2s (Sca-$1^+$c-$kit^{hi}CD135^-CD48^+CD150^-$).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5.0 (GraphPad Software). The details of the test carried out are indicated in the figure legends. Data are represented as means ± SEM and distributed approximately normally. Data were analyzed using one-way ANOVA and Tukey's or Dunnett's multiple comparison test, two-way ANOVA and Bonferroni posttest, or Student's *t* test (two-tailed). Significance of differences in Kaplan-Meier survival curves was determined using the log-rank test (two-tailed). In all cases, $P < 0.05$ was considered significant.

SUPPLEMENTARY MATERIALS

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Materials and Methods
Fig. S1. TKI treatment induces CD70 expression on human BCR-ABL1$^+$ leukemia cell lines.
Fig. S2. TKI treatment mediates CD70 induction via BCR-ABL1 inhibition.
Fig. S3. CD70 up-regulation is induced early after TKI treatment in KBM5r cells.
Fig. S4. Activation of Wnt signaling by lithium chloride or R-Spondin 1 restores TKI-mediated changes in gene expression.
Fig. S5. BCR-ABL1 and CD70/CD27 co-inhibition reduces the expansion of KBM5 and KBM5r CML cells in vitro.
Fig. S6. BCR-ABL1 and CD70/CD27 co-inhibition synergistically reduces cell growth and Wnt pathway activation in SD-1 cells.
Fig. S7. Combination treatment only marginally affects "healthy donor" BM stem/progenitor cells.
Fig. S8. Imatinib concentration was measured in the plasma of xenografted CML mice.
Fig. S9. CD70 and CD27 expression were determined on primary human CML cells.
Fig. S10. CD70 expression on murine LSCs and endogenous nonmalignant GFP$^-$ LSKs was evaluated after imatinib treatment.
Fig. S11. TKI treatment induces CD70 expression on murine LSCs but not on leukemia progenitors or endogenous nonmalignant GFP$^-$ LSKs.
Fig. S12. Combination therapy eradicates LSCs and promotes long-term survival of CML mice.
Table S1. Patient characteristics.
Table S2. Synergistic growth inhibition of SD-1 cells by targeting BCR-ABL1 and CD27 signaling.
Table S3. List of primers.

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Tyrosine kinase inhibitor–induced CD70 expression mediates drug resistance in leukemia stem cells by activating Wnt signaling

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A one-two punch for CML

Chronic myelogenous leukemia (CML) is classically associated with the pathogenic BCR-ABL1 translocation, which can be targeted by treatment with imatinib and other tyrosine kinase inhibitors. Unfortunately, these treatments do not eradicate leukemia stem cells, and many patients eventually relapse. Now, Riether *et al*. have identified a mechanism for treatment resistance in this cancer, showing that it can be caused by up-regulation of CD70 and resulting up-regulation of the Wnt pathway. In addition, the authors showed that this resistance can be successfully blocked by inhibiting the activity of CD70, suggesting a clinical application for this finding.