

LYMPHOID NEOPLASIA

Integrin $\alpha 6$ mediates the drug resistance of acute lymphoblastic B-cell leukemia

Eun Ji Gang,¹ Hye Na Kim,¹ Yao-Te Hsieh,¹ Yongsheng Ruan,¹ Heather A. Ogana,¹ Solomon Lee,¹ Jennifer Pham,¹ Huimin Geng,² Eugene Park,¹ Lars Klemm,² Cheryl L. Willman,⁴ William L. Carroll,⁵ Steven D. Mittelman,⁶ Etan Orgel,¹ Matthew J. Oberley,³ Chintan Parekh,¹ Hisham Abdel-Azim,¹ Deepa Bhojwani,¹ Alan S. Wayne,¹ Adèle De Arcangelis,⁷ Elisabeth Georges-Labouesse,⁷ Elizabeth Wayner,⁸ Halvard Bonig,^{9,10} Aspram Minasyan,¹¹ Johanna ten Hoeve,¹¹ Thomas G. Graeber,¹¹ Markus Müschen,² Nora Heisterkamp,² and Yong-Mi Kim¹

¹Department of Pediatrics, Division of Hematology-Oncology, Children's Hospital Los Angeles, Norris Comprehensive Cancer Center, University of Southern California Keck School of Medicine, Los Angeles, CA; ²Department of Systems Biology, Beckman Research Institute at City of Hope, Duarte, CA; ³Department of Pathology and Laboratory Medicine, Children's Hospital Los Angeles, University of Southern California Keck School of Medicine, Los Angeles, CA; ⁴University of New Mexico Cancer Center and Departments of Pathology, Internal Medicine, Mathematics and Statistics, and Physics and Astronomy, University of New Mexico, Albuquerque, NM; ⁵Department of Pathology, New York University School of Medicine, New York, NY; ⁶Division of Pediatric Endocrinology, University of California Los Angeles (UCLA) Children's Discovery and Innovation Institute, David Geffen School of Medicine, UCLA, Los Angeles, CA; ⁷Institut de Génétique et de Biologie Moléculaire et Cellulaire, Centre National de la Recherche Scientifique (CNRS) Unité Mixte de Recherche (UMR) 7104, INSERM U1258, Université de Strasbourg, Illkirch, France; ⁸Fred Hutchinson Cancer Center, University of Washington, Seattle, WA; ⁹Goethe University School of Medicine, Institute for Transfusion Medicine and Immunohematology and German Red Cross Blood Service BaWuHe, Frankfurt, Germany; ¹⁰Department of Medicine/Hematology, University of Washington, Seattle, WA; and ¹¹Department of Molecular and Medical Pharmacology, UCLA Metabolomics Center, UCLA, Los Angeles, CA

KEY POINTS

- Integrin $\alpha 6$ -mediated adhesion promotes the survival of chemotherapy-treated B-ALL.
- Integrin $\alpha 6$ is evaluated as a therapeutic target for B-ALL.

Resistance to multimodal chemotherapy continues to limit the prognosis of acute lymphoblastic leukemia (ALL). This occurs in part through a process called adhesion-mediated drug resistance, which depends on ALL cell adhesion to the stroma through adhesion molecules, including integrins. Integrin $\alpha 6$ has been implicated in minimal residual disease in ALL and in the migration of ALL cells to the central nervous system. However, it has not been evaluated in the context of chemotherapeutic resistance. Here, we show that the anti-human $\alpha 6$ -blocking Ab P5G10 induces apoptosis in primary ALL cells in vitro and sensitizes primary ALL cells to chemotherapy or tyrosine kinase inhibition in vitro and in vivo. We further analyzed the underlying mechanism of $\alpha 6$ -associated apoptosis using a conditional knockout model of $\alpha 6$ in murine BCR-ABL1⁺ B-cell ALL cells and showed that $\alpha 6$ -deficient ALL cells underwent apoptosis. In vivo deletion of $\alpha 6$ in combination with tyrosine kinase inhibitor (TKI) treatment was more effective in eradicating ALL than treatment with a TKI (nilotinib) alone. Proteomic analysis revealed that $\alpha 6$ deletion in murine ALL was associated with changes in Src signaling, including the upregulation of phosphorylated Lyn (pTyr507) and Fyn (pTyr530). Thus, our data support $\alpha 6$ as a novel therapeutic target for ALL. (*Blood*. 2020;136(2):210-223)

Introduction

Despite much progress over the last several decades, the overall survival of patients with acute lymphoblastic leukemia (ALL) has plateaued at approximately 40% for adults and approximately 90% for children.^{1,2} Bone marrow (BM) is the most frequent site of relapse for ALL,^{3,4} and BM relapse is associated with a worse prognosis than isolated extramedullary relapse.^{3,5} In vitro studies show that contact of leukemia cells with stromal cells promotes cell adhesion-mediated drug resistance (CAM-DR),^{6,7} which prevents the apoptosis of ALL cells⁸⁻¹⁰ and contributes to the survival of ALL cells.¹¹ The term minimal residual disease (MRD) refers to a situation in which clinical remission has been achieved, but residual leukemia cells remain detectable by flow cytometry or polymerase chain reaction (PCR) assays.^{12,13} The identity of the adhesion molecules that mediate CAM-DR to sustain MRD despite treatment remains elusive.

Integrins, a family of glycoprotein adhesion cell surface receptors composed of α and β subunits,^{14,15} are critical for cell adhesion to the extracellular matrix (ECM) in the BM environment. Hematopoietic stem cells (HSCs) bind via integrin $\alpha 6$, also known as CD49f or Itga6 and hereafter called $\alpha 6$, to several isoforms of the ECM protein laminin, a heterotrimer of α , β , and γ chains.¹⁶ Notta et al¹⁶ showed that high expression of $\alpha 6$ indicates the presence of immature HSCs in cord blood. By using proteomics and transcriptomics approaches, $\alpha 6$ was identified as a leukemic stem cell (LSC) marker in acute myeloid leukemia (AML),¹⁷ and $\alpha 6$ was associated with drug resistance in AML.¹⁸ In ALL, $\alpha 6$ was detected by flow cytometry in B-cell ALL (B-ALL) patients¹⁹ and has been proposed for addition to the MRD flow cytometry marker panel for ALL.¹³ Importantly, $\alpha 6$ -laminin interactions mediate the migration of ALL cells toward the cerebrospinal fluid in vitro, and xenografts of 2 B-ALL cell lines treated with a commercially available $\alpha 6$ integrin-neutralizing antibody (Ab)

showed reduced central nervous system involvement.²⁰ These findings indicate the need to functionally analyze the role of $\alpha 6$ in B-ALL in the context of resistance to chemotherapy. We hypothesized that $\alpha 6$ represents more than a disease biomarker, and therefore, we proceeded to study the effects of the functional loss of $\alpha 6$ in genetic and pharmacologic models of patient-derived (primary) B-ALL to assess whether $\alpha 6$ can be targeted to eradicate ALL.

Methods

Correlation of $\alpha 6$ gene expression on leukemic blasts with the clinical outcomes of B-ALL patients

Clinical and gene expression microarray data from 207 high-risk B-precursor ALL patients from the Children's Oncology Group (COG) Clinical Trial P9906 were obtained from the Gene Expression Omnibus database (GSE11877).²¹ The patients were treated uniformly with a modified augmented Berlin-Frankfurt-Münster Study Group regimen, and individuals with very high-risk features (*BCR-ABL1* or hypodiploidy) were excluded from the study. Cryopreserved residual pretreatment leukemia specimens were available for a representative cohort of 207 patients, including 131 BM and 76 peripheral blood (PB) samples. RNA was purified from these pretreatment diagnostic samples, which contained more than 80% blasts. The majority of patients ($n = 191$) had MRD, as assessed by flow cytometry; patients were defined as MRD-positive or MRD-negative at the end of induction therapy (day 29) using a threshold of 0.01% (the presence of $\geq 0.01\%$ or $< 5\%$ ALL cells was defined as MRD). The comparison of $\alpha 6$ expression in the MRD-positive and MRD-negative patient groups was performed using the Wilcoxon test in the R package (R Development Core Team; <http://www.R-project.org/>).

Patient samples

BM and PB samples from ALL patients were acquired in compliance with the institutional review board regulations of each institution. Informed consent for cell banking was obtained from all human patients. Leukemia cells were processed and cultured as previously described.²² Human studies were conducted in accordance with the Declaration of Helsinki.

De-adhesion of primary ALL and murine $\alpha 6^{fl/fl}$ p210 cells

Primary B-ALL cells were cultured on human laminin (hlaminin) or murine OP9 stroma cells for 4 hours at 37°C in 5% CO₂ and subsequently incubated overnight with anti- $\alpha 6$ Ab (clone P5G10, a gift from Elizabeth Wayner) or isotype control immunoglobulin G1 (IgG1) (BioLegend). Cells suspended in the supernatant were removed. Adherent cells were detached by pipetting with phosphate-buffered saline (PBS) and counted after trypan blue exclusion staining of dead cells. Murine $\alpha 6^{fl/fl}$ BCR-ABL1⁺p210 EmptyER^{T2} and CreER^{T2} cell production is described in the supplemental Data (available on the *Blood* Web site). Murine cells were treated with tamoxifen for the indicated number of days to induce deletion followed by plating and incubation of the cells on murine laminin (mlaminin) overnight.

In vitro drug assay

Human B-ALL cells plated on hlaminin-1 or OP9 cells were treated with vincristine, dexamethasone, and L-asparaginase (VDL) (vincristine; Hospira, Inc; dexamethasone, Fresenius Kabi USA; and L-asparaginase, KRS Global Technologies) at the

indicated concentrations for indicated times, with anti- $\alpha 6$ Ab P5G10 (20 $\mu\text{g}/\text{mL}$),²³ J8H (20 $\mu\text{g}/\text{mL}$) (Sanquin Blood Supply Foundation, Amsterdam, The Netherlands), or IgG1 control Ab (BioLegend). BCR-ABL⁺ ALL was treated with nilotinib at the indicated concentrations. Murine $\alpha 6^{fl/fl}$ BCR-ABL1⁺p210 EmptyER^{T2} and CreER^{T2} cells cultured on mlaminin were treated with tyrosine kinase inhibitor (TKI) nilotinib (0.02 μM or 0.2 μM) for 4 days. Viability was determined by using trypan blue exclusion staining; annexin V/7-aminocincomycin D (7-AAD) or propidium iodide staining was performed as indicated. All cells were harvested from the wells, and the stromal cells were distinguished from the leukemia cells by their characteristic shape and larger sizes under a light microscope.

PCR and qRT-PCR

Genomic DNA from $\alpha 6^{fl/fl}$ p210 EmptyER^{T2} or CreER^{T2} cells at 6 days after tamoxifen (Sigma-Aldrich) treatment was extracted using the DNeasy Blood & Tissue Kit (QIAGEN). For quantitative reverse transcription PCR (qRT-PCR), total RNA was extracted using RNeasy Plus Micro and Mini Kits (QIAGEN) and reverse-transcribed into complementary DNA (cDNA) by SuperScript III (Invitrogen). qRT-PCR was performed with an ABI7900HT real-time PCR system (Applied Biosystems). Primers are listed in supplemental Table 2.

Flow cytometry

Primary B-ALL cells were stained with Abs against CD19, CD45, and CD49f (BD Biosciences). Murine $\alpha 6^{fl/fl}$ p210 EmptyER^{T2} and CreER^{T2} cells were stained with Abs against murine CD19, CD45, and CD49f (eBioscience). Apoptosis or cell viability was analyzed with annexin V-phycoerythrin (PE) or 7-AAD staining (BD Biosciences). Cell cycle analysis was performed using anti-bromodeoxyuridine (BrdU) allophycocyanin Abs. BrdU incorporation and DNA content (7-AAD) were measured in fixed and permeabilized cells (BD Biosciences). Abs and isotype controls are listed in supplemental Table 3.

siRNA transfection

Transfection with Lyn-, Fyn-, or Csk-specific or negative control ON-TARGETplus short interfering RNA (siRNA; scrambled siRNA [Scr]; Dharmacon) (50 nM) was performed using DharmaFect reagent according to the manufacturer's instructions. The siRNAs were added to the cell culture media for the indicated times. Western blot analysis (data are shown for Csk siRNA but not for Lyn or Fyn siRNA) was performed to confirm the knockdown effects of the siRNAs on gene expression at 48 hours after transfection.

Western blotting

Cells were lysed in Mammalian Protein Extraction Reagent (M-PER) buffer (Thermo Fisher Scientific) supplemented with a 1% protease inhibitor cocktail (Thermo Fisher Scientific), and the proteins were separated by 4% to 12% sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrotransferred to polyvinylidene difluoride membranes (Invitrogen) as described previously.²⁴ The Abs are listed in supplemental Table 3.

Immunohistochemistry

BM biopsies from the patients were immersed in 4% paraformaldehyde (Sigma-Aldrich) for 4 hours and transferred into PBS before being embedded in paraffin. The 5- μm sections were stained with Abs against CD79a (Leica Biosystems) and CD49f/ $\alpha 6$ (Abcam) and visualized by a Bond Polymer Refine

Detection Kit (Leica Microsystems). The details are described in the supplemental Data. The Abs used are listed in supplemental Table 3.

Phosphoproteomic profiling of phosphotyrosines by high-pressure liquid chromatography

The phosphotyrosine proteomics analysis sample preparation was performed as described previously²⁵ with some modifications that are described in the supplemental Data.

In vivo mobilization of human primary ALL cells

Primary ALL cells (2.5×10^6 cells per mouse) were injected intravenously into NOD.Cg-Prkdc^{scid}/Il2rg^{tm1Wjl}/SzJ (NSG) mice (The Jackson Laboratory). The mice then received 2 injections of 30 mg/kg P5G10, PBS, or the IgG1 control intravenously or intraperitoneally on day 27 and day 29 (LAX7R), day 38 and day 40 (TXL3), and day 18 and day 20 (PDX2) after ALL injection. The percentages of human CD45⁺ and CD19⁺ cells in the PB were analyzed by flow cytometry²² before, and at 1 and 3 days after P5G10 treatment. All animal studies were approved by the Institutional Animal Care and Use Committee at Children's Hospital Los Angeles.

In vivo treatment of human ALL cells with anti-human $\alpha 6$ Ab (P5G10) and chemotherapy

Primary ALL LAX7R cells labeled with firefly luciferase (LUC) by transduction with pCCL-MNDU3-LUC viral supernatant were intravenously injected into sublethally irradiated (250 cGy) NSG mice (0.05×10^6 cells per mouse).²⁶ Vincristine (0.5 mg/kg) was administered once per week, and dexamethasone (10.5 mg/kg) and L-asparaginase (1500 IU/kg) were administered intraperitoneally 5 days per week.²⁷ P5G10 (30 mg/kg) was administered intravenously once per week for 4 weeks.

In vitro and in vivo models of BCR-ABL1-transformed $\alpha 6^{fl/fl}$ ALL cells and bioluminescence imaging

Mononuclear cells (MNCs) from whole BM of $\alpha 6^{fl/fl}$ mice were isolated by red blood cell lysis using BD Lysis Buffer and were retrovirally transduced with BCR-ABL1 expression vectors²⁵ in the presence of 10 ng/mL murine interleukin-7 (Peprotech) on retro-nectin (Takara Bio USA)-coated plates. After cytokine-independent proliferation, the BCR-ABL1-transformed $\alpha 6^{fl/fl}$ cells were transduced retrovirally with EmptyER^{T2} or CreER^{T2} followed by selection with puromycin (1 μ g/mL) (Invitrogen). After selection with 1 μ g/mL puromycin, $\alpha 6$ deletion was induced in vitro by the addition of 1.5 μ M tamoxifen (Sigma-Aldrich). For the in vivo deletion of $\alpha 6$, 0.5×10^6 luciferase-labeled $\alpha 6^{fl/fl}$ BCR-ABL1⁺ EmptyER^{T2} or CreER^{T2} ALL cells were injected via the tail vein into sublethally irradiated (250 cGy) NOD/SCID mice. $\alpha 6$ in vivo deletion was induced by daily administration of 100 to \sim 150 mg/kg tamoxifen via oral gavage on days 3 to 8 and days 16 to 20 after leukemia cell transfer. For bioluminescent imaging, these cells were also transduced with a lentiviral vector (pCCL-MNDU3-LUC) encoding firefly luciferase and a neomycin selection marker in 24-well plates coated with retronectin (Takara Bio USA) for 48 hours as described previously.⁶ Additional details are provided in the supplemental Data.

Microarray and statistical analyses

Itga6 expression was analyzed in murine B-cell progenitor cells using ImmGen (http://rstats.immgen.org/Skyline_microarray/skyline.html). A Student t test was performed to compare the

differences between 2 groups for the in vitro studies. One-way analysis of variance with post hoc analysis (Tukey test) was used to compare multiple groups. The log-rank test was used to evaluate differences in the median survival time (MST) for the in vivo studies. A value of $P < .05$ indicated a significant difference.

Results

High expression levels of $\alpha 6$ at the time of diagnosis predict positive MRD on day 29

To determine the significance of integrin $\alpha 6$ expression in ALL patients, we correlated the expression of $\alpha 6$ with clinical outcomes in 191 high-risk (excluding BCR/ABL1⁺ patients) B-ALL patients uniformly treated according to the regimen used by the COG P9906 clinical trial.²¹ Patients with ALL blasts with $\alpha 6^{\text{high}}$ expression at diagnosis were less likely to achieve deep remission by day 29 (MRD-positive) than patients with ALL blasts with $\alpha 6^{\text{low}}$ expression (MRD-negative) ($P = .0005$ and $P = .001$, respectively, for the 2 $\alpha 6$ probe sets) (Figure 1A). Interestingly, we found that BM biopsies with MRD-positive but not MRD-negative samples revealed $\alpha 6$ costaining with CD79a as a marker of B-lineage cells, confirming $\alpha 6$ as a marker of MRD in primary samples (Figure 1B). Cell surface expression of $\alpha 6$ was further analyzed by flow cytometry for 10 patient-derived (primary) B-ALL cells from patients with different karyotypes (supplemental Table 1). Virtually all ALL samples were $\alpha 6^+$ (Figure 1C), but quantitative differences in mean fluorescence intensity were noted (Figure 1D). In contrast, CD19⁺ B cells from healthy BM donors had relatively low expression of $\alpha 6$. To examine this in more detail, we analyzed messenger RNA (mRNA) expression using ImmGen-Skyline with a data set from normal murine progenitor cells and found expression of $\alpha 6$ in B-lineage precursors (supplemental Figure 1A). We also performed more detailed flow cytometry analysis confirming the low expression of $\alpha 6$ on BM-derived murine precursor B cells of C57Bl6/J mice (mCD19⁺B220⁺CD25⁺CD43⁺)²⁸ ($8.17\% \pm 0.18\%$) ($n = 2$) and on normal human precursor B cells (hCD19⁺CD45⁺CD20-IgM-CD34-CD10⁺)²⁹ ($3.24\% \pm 1.21\%$) ($n = 3$) (supplemental Figure 1B-D).

Integrin $\alpha 6$ blockade deadheres B-ALL cells from their ligand laminin-1 and stromal cells

To evaluate whether blocking $\alpha 6$ -mediated adhesion can overcome CAM-DR, we first analyzed the adhesion of B-ALL cells to several laminins, which are ligands of $\alpha 6$, using an anti- $\alpha 6$ blocking Ab approach. We determined that 20 μ g/mL P5G10²³ saturates binding of $\alpha 6$ in primary ALL cells (supplemental Figure 2). A concentration of 50 μ g/mL laminin-1, which is a specific ligand of $\alpha 6$, stimulated the adherence of approximately 90% of ALL cells (supplemental Figure 3); higher concentrations were not tested. We found that P5G10 deadhered ALL cells from 3 different laminin isoforms, namely, laminin-111 (hereafter referred to as laminin-1), laminin-511 (also known as laminin-10), and laminin-521 (also known as laminin-11) (supplemental Figure 4A-B), all of which are expressed in normal human BM.³⁰ Because the level of deadhesion from different laminin isoforms was similar, laminin-1 was used for subsequent studies. Five primary ALL cell lines (LAX7R, LAX53, PDX2, TXL3, and SFO2) with different karyotypes (supplemental Table 1) were plated onto laminin-1 or OP9 cells. Murine stroma-derived OP9 cells were chosen because they support the viability and growth of primary ALL cells.²² The expression of laminin was confirmed by western blotting (supplemental Figure 4C). The P5G10 Ab²³ deadhered all

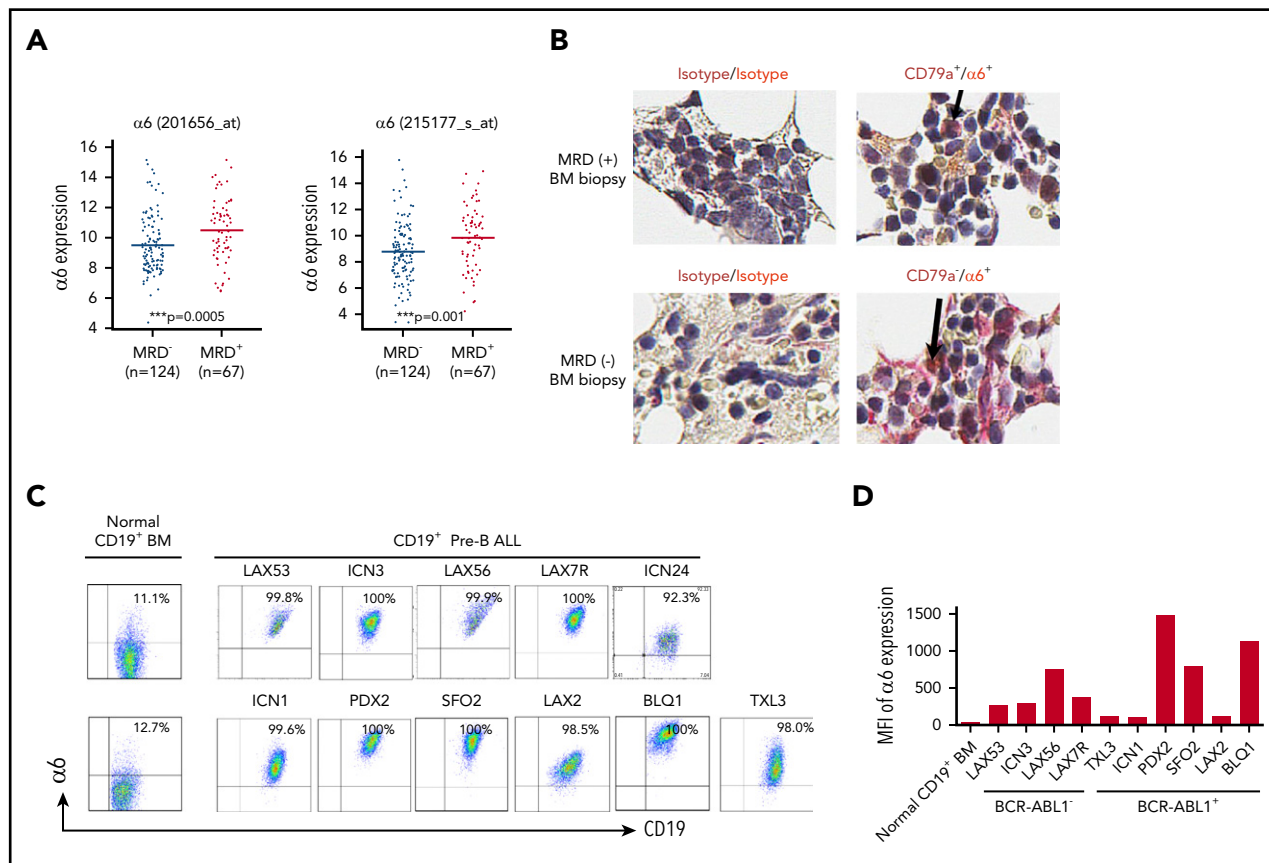


Figure 1. Integrin $\alpha 6$ is highly expressed in primary B-ALL. (A) Two probe sets for integrin $\alpha 6$ (*ITGA6*) in patients with MRD⁺ and MRD⁻ ALL from COG study P9906 were analyzed. MRD was measured by flow cytometry at the end of induction therapy (day 29) using patients' BM or PB samples. RNA was purified from 191 pretreatment diagnostic samples with more than 80% blasts (131 BM; 76 PB). The horizontal bars indicate the average levels of integrin $\alpha 6$ expression. *P* values were obtained by using the Wilcoxon test for each probe set. ****P* = .0005 for probe 201656_at; ****P* = .001 for probe 215177_s_at. (B) Immunohistochemical staining of isotype controls (left panel) or CD79a (brown) and $\alpha 6$ (red) expression (right panel) in MRD⁺ and MRD⁻ biopsy specimens. Original magnification, $\times 630$. (C) Representative dot plots of cell-surface $\alpha 6$ expression in B-ALL and normal BM CD19⁺ B-cell samples. One of 3 experiments is shown. (D) Representative mean fluorescence intensity (MFI) of $\alpha 6$ expression in CD19⁺ B-ALL and normal BM CD19⁺ B cells. One of 3 experiments is shown.

8 ALL cell lines from laminin-1, unlike the control-treated ALL cells, but was less effective at deadhering the ALL cells from OP9 (Figure 2A-E). These experiments indicate that adhesion to OP9 cells is mediated through additional adhesion molecules besides $\alpha 6$ but that $\alpha 6$ is a relevant laminin receptor on ALL cells.

To determine the effect of the inhibition of $\alpha 6$ function on the ability of ALL cells to resist drug treatment, 6 primary B-ALL cell lines (LAX7R, LAX53, PDX2, TXL3, SFO2, ICN24) and 2 B-ALL cell lines (Kasumi-2 and BEL-1) were plated on bovine serum albumin control or laminin-1 and then treated for 2 days with VDL or with nilotinib in Philadelphia chromosome-positive (Ph⁺) ALL (BCR-ABL1⁺) with or without P5G10 or its control isotype Ab (IgG1). Four of the 8 ALL cell lines had already shown decreased viability after treatment with P5G10 alone (Figure 2 F,H-J,K-M). Cells from all 8 patients with ALL showed statistically significant but moderate decrease in viability when P5G10-mediated $\alpha 6$ blockade was combined with VDL or TKI treatment compared with treatment with VDL or TKI alone (Figure 2 F-J). P5G10 treatment also decreased the viability of ALL cells plated on murine stroma OP9 cells treated in combination with chemotherapy (VDL) or nilotinib (Figure 2 N-P).

To determine whether the physical deadhesion associated with P5G10 induces apoptosis or whether apoptosis is independent

of deadhesion, we used J8H, which is another anti-human $\alpha 6$ signaling function-blocking Ab.³¹ LAX7R ALL cells that were adherent to laminin-1 or OP9 cells were almost fully deadhered by P5G10 and were markedly less deadhered by J8H (Figure 2Q), but both anti- $\alpha 6$ Abs decreased the viability of ALL cells compared with that of controls (Figure 2R), indicating that $\alpha 6$ -associated apoptosis is induced with 2 different $\alpha 6$ Abs and is partly independent of deadhesion.

Integrin signaling has previously been shown to promote the survival of pre-B-ALL cells by modulating PI3K signaling and the expression of apoptotic regulators, including Bcl2, caspases 3 and 7, and IAP family members.³² To identify the mechanism that underlies apoptosis associated with integrin $\alpha 6$ blockade, LAX7R cells were treated with P5G10 or IgG1 for 1 hour before 48 hours of exposure to VDL or vehicle control (supplemental Figure 5 A-B). Interestingly, the levels of the antiapoptotic IAP family member survivin²⁶ were increased by chemotherapy treatment but reduced by P5G10 treatment, and the levels of cleaved caspase 3 or 7 were not (supplemental Figure 5C).

Integrin $\alpha 6$ blockade eradicates leukemia when combined with chemotherapy in vivo

Next, we determined whether P5G10 can restore the chemotherapy sensitivity of leukemia cells in vivo. NSG mice were

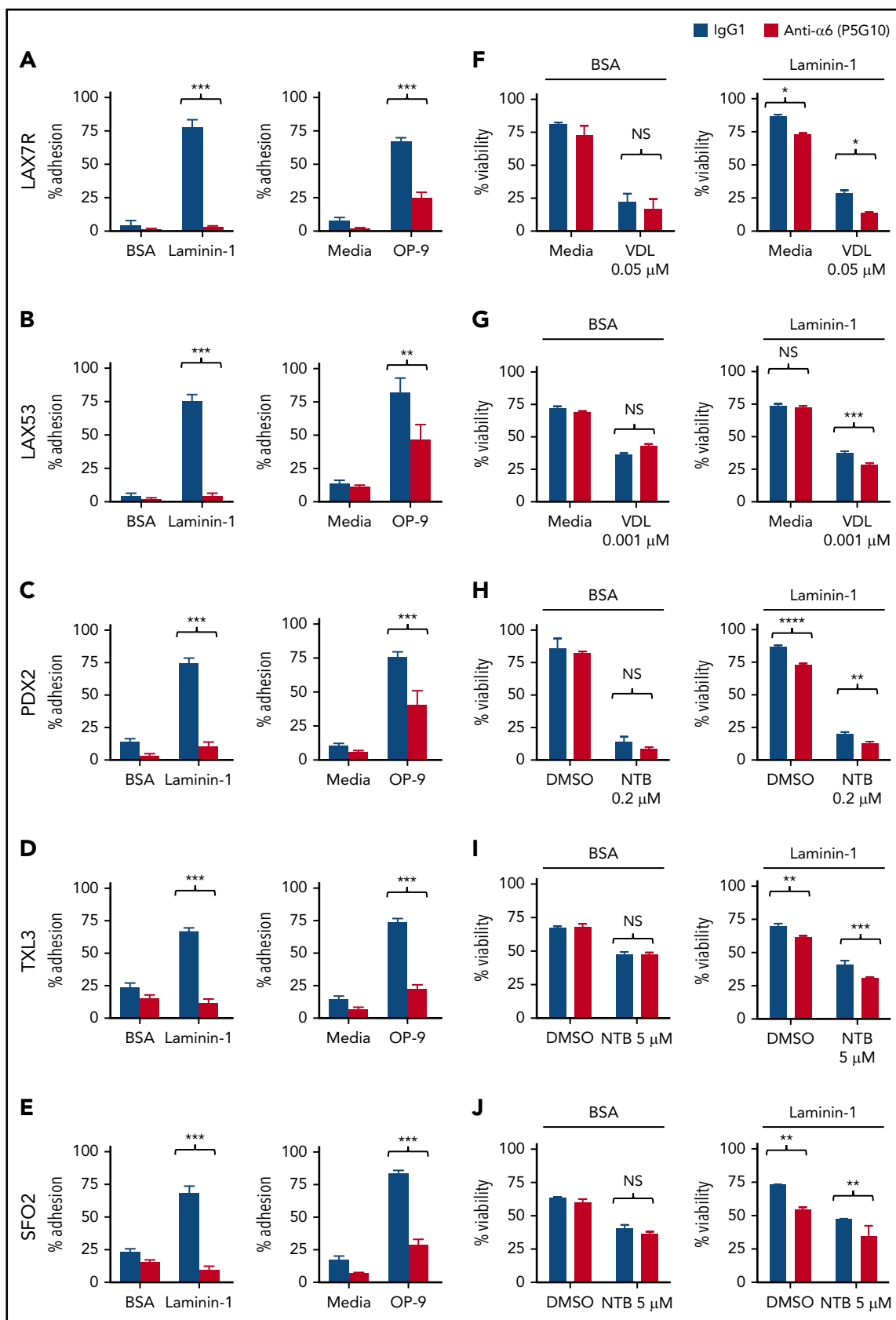


Figure 2. Integrin $\alpha 6$ blockade using the anti- $\alpha 6$ mAb P5G10 deadheres primary ALL cells and sensitizes the cells to chemotherapy. Six primary B-ALL cell lines (LAX7R, LAX53, PDX2, TXL3, SFO2, ICN24) and 2 B-ALL cell lines (Kasumi-2 and BEL-1) were preincubated with purified anti-human $\alpha 6$ Ab P5G10 (red bars) or its isotype control IgG1 (white bars) on plates coated with laminin-1. Adhesion of ALL cells to the bovine serum albumin (BSA) control, laminin-1 (A-E, left panels), cell culture media (media) or OP9 stroma cells (A-E, right panels) was assessed after overnight incubation. The viability of the ALL cells LAX7R (F), LAX53 (G), PDX2 (H), TXL3 (I), SFO2 (J), and ICN24 (K), plated on

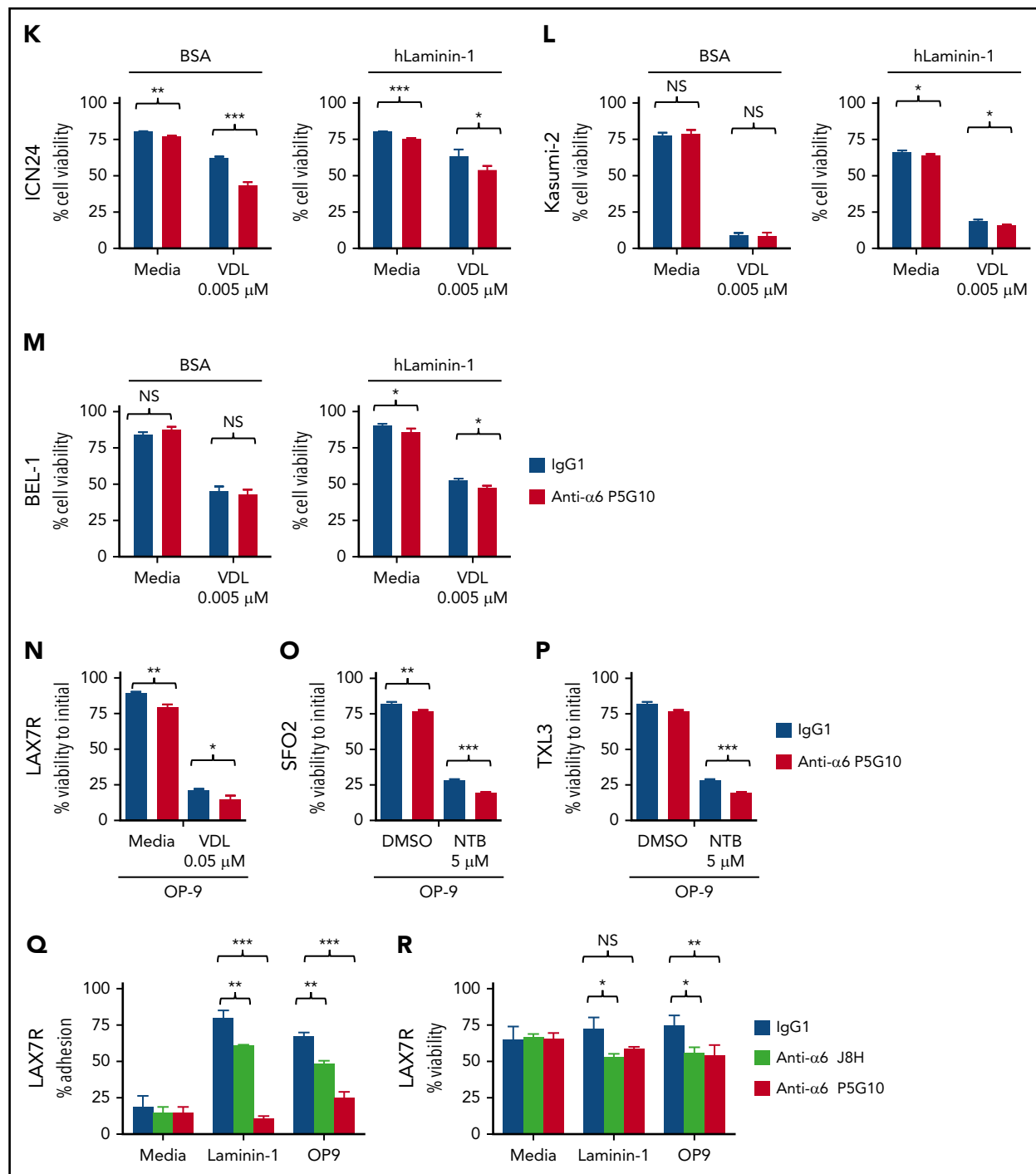


Figure 2 (continued) BSA (left panel) or laminin-1 (right panel) and treated with or without $\alpha 6$ blockade (P5G10) combined with nilotinib (NTB) or VDL for 2 days is shown. Viability of B-ALL cell lines Kasumi-2 (L) and BEL-1 cells (M) plated on BSA (left panel) or laminin-1 (right panel) treated with VDL combined with P5G10 for 3 days is shown. Three primary ALL cell lines, LAX7R (KRAS^{G12V}) (N), SFO2 (BCR-ABL1⁺) (O), and TXL3 (BCR-ABL1⁺) (P), were cocultured with OP9 cells and incubated with P5G10 (red bars) or its isotype control IgG1 (white bar) combined with NTB or VDL for 5 days. VDL was used at indicated concentrations (0.005 μ M: 5 nM vincristine, 50 pM dexamethasone, and 0.0025 IU L-asparaginase; 0.001 μ M: 1 nM vincristine, 10 pM dexamethasone, and 0.005 IU L-asparaginase; 0.05 μ M: 50 nM vincristine, 500 pM dexamethasone, and 0.025 IU L-asparaginase). Viability was measured via the exclusion of dead cells based on trypan blue staining. LAX7R cells (Q-R) were plated on laminin-1 or OP9 cells and treated with the following antibodies: $\alpha 6$ function-blocking J8H (gray bars; 20 μ g/mL), $\alpha 6$ adhesion- and function-blocking P5G10 (red bars; 20 μ g/mL), or IgG1 (white bar; 20 μ g/mL). Adhesion (overnight incubation) (Q) and viability (2 days incubation) (R) were determined by trypan blue exclusion staining. Means \pm standard deviations are shown. One experiment of 3 is shown. Each experiment was performed in triplicate. * $P < .05$; ** $P < .01$; *** $P < .001$ by 1-way analysis of variance (ANOVA) with post hoc analysis (Tukey test). DMSO, dimethyl sulfoxide; NS, not significant.

treated 3 days after the injection of luciferase-labeled LAX7R cells with 4 once-per-week injections of anti-human $\alpha 6$ P5G10 monoclonal Ab (mAb) or saline with or without VDL (Figure 3A).

Mice treated with P5G10 alone survived at rates similar to those of control mice (PBS MST, 39 days vs P5G10 MST, 31 days; $P = .65$) (Figure 3B). Mice treated with chemotherapy relapsed

shortly after the end of treatment and succumbed to leukemia. In marked contrast, 2 of the 5 mice treated with a combination of chemotherapy and P5G10 survived and were apparently disease free ($P = .004$ compared with VDL-only treatment). In vivo bioluminescence assays also demonstrated a significant decrease in leukemia progression induced by the combination of P5G10 and VDL compared with that induced by VDL alone (Figure 3C). Three animals treated with the combination that succumbed to leukemia or were euthanized on day 185 or day 186 after leukemia injection had BM that was negative for human CD19, according to flow cytometry (Figure 3D). These data indicate that specific treatment with the P5G10 Ab combined with chemotherapy could significantly prolong the survival of leukemic mice and had the potential to induce long-term remission. To exclude nonspecific effects of Abs, different control treatments (PBS, Ig, and IgG1) were administered intraperitoneally once per week for 2 weeks to leukemic mice. Leukemia progression monitored by bioimaging and weight development were similar among control groups (supplemental Figure 6A-D).

Integrin $\alpha 6$ blockade has the potential to cause modest mobilization of leukemia cells to PB

Integrin blockade can mobilize ALL cells to migrate to the PB, as shown by integrin $\alpha 4$ inhibition in ALL.²² To determine whether $\alpha 6$ blockade functionally induced the mobilization of leukemia cells to migrate to the PB, TXL3 and PDX2 (both BCR-ABL1⁺) and LAX7R (KRAS^{G12V}) patient-derived ALL cells were injected into NSG mice. On day 1 after treatment with P5G10, 2 (LAX7R, PDX2) of 3 mice treated with P5G10 did not show an increase in leukemia cells in the PB compared with the number present before P5G10 treatment (day 0) or compared with the number in control recipient mice (supplemental Figures 7 and 8), 1 of 3 mice did (TXL3). On day 3 after treatment with P5G10, 1 (LAX7R) of 3 mice treated with P5G10 did not show an increase in leukemia cells in the PB (supplemental Figures 7 and 8), but 2 of 3 mice (TXL3, PDX2) did show modest mobilization of leukemia cells to the PB.

$\alpha 6$ deletion deadheres BCR-ABL1⁺ leukemia cells and induces their apoptosis

To further investigate the pro-survival function of $\alpha 6$, we made use of BCR-ABL1 (p210) ERT² of $\alpha 6^{fl/fl}$ B-ALL cells (supplemental Figures 9 and 10), in which the $\alpha 6$ protein can be ablated by tamoxifen treatment. When ERT²-Cre x $\alpha 6^{fl/fl}$ B-ALL cells were treated with tamoxifen, $\alpha 6$ expression was lost, as measured by flow cytometric analysis of $\alpha 6$ surface expression (Figure 4A-B) and genomic PCR (Figure 4C). This loss corresponded to cell cycle inhibition, as shown by the decreased percentage of cells in S-phase and the increased percentage of cells in the G₀/G₁ and G₂/M phases compared with that in $\alpha 6$ WT cells (Figure 4D). In concordance with this result, deletion of $\alpha 6$ also caused decreases in proliferation (supplemental Figure 11A-B). As a non- $\alpha 6^{fl/fl}$ gene control, we previously showed in a conditional knockout model of murine integrin $\alpha 4^{fl/fl}$ BCR/ABL1⁺ CreERT² leukemia that tamoxifen treatment did not lead to apoptosis in vitro,²² and we have repeated this experiment in vitro (supplemental Figure 12A-B) to demonstrate that Cre activation in $\alpha 4^{fl/fl}$ cells does not cause apoptosis. Therefore, Cre deletion is not generally associated with apoptosis induced from deletion-induced toxicity, supporting the conclusion that $\alpha 6$ deletion is specifically associated with apoptosis.

Adhesion was significantly decreased in $\alpha 6$ -deleted cells compared with that in non-deleted control cells by day 5 after tamoxifen treatment (Figure 4E). We observed a further decrease in the cell viability of $\alpha 6$ -deleted leukemia cells, as determined by flow cytometry (Figure 4F). Western blotting of $\alpha 6$ -deleted cells compared with that of $\alpha 6$ -competent cells showed a marked increase in apoptotically cleaved caspases 3, 7, 8, and 9 on day 5 after tamoxifen treatment (Figure 4G). When we analyzed the time course of $\alpha 6$ -deleted cells on days 4, 5, and 6 after tamoxifen-induced deletion (Figure 4H), we observed progressive apoptosis over time (Figure 4I) with an increase in cleaved poly (ADP-ribose) polymerase and p53 expression (Figure 4J).

$\alpha 6$ deletion is associated with Src signaling changes in BCR-ABL1⁺ leukemia

Few studies have focused on signal transduction downstream of integrin $\alpha 6$. Because there is an abundance of literature on Src family kinases and other tyrosine kinases, which play important roles in the signaling of integrins,³⁴⁻³⁸ we investigated specific changes in total tyrosine phosphorylation in $\alpha 6$ -deleted cells. As shown in Figure 5A, cells deleted for $\alpha 6$ showed increased signaling from pTyr-containing proteins of 50 to 60 kDa (red lines) within 24 hours compared with empty vector x $\alpha 6^{fl/fl}$ B-ALL cells. To identify the affected proteins, we performed proteomic pTyr analysis and identified increased tyrosine phosphorylation of the docking protein CASL, which assembles signal transduction complexes that include integrins and decreases the phosphorylation of specific Y residues in Src family kinase substrates³⁹ such as SHIP2 (Y623) (at the 16-hour time point), GSK3 β (Y216)⁴⁰ (at both the 16-hour and 3-day time points), Abl (Y413), Crkl (Y92), and Stat5A (90Y) (Figure 5B). Interestingly, increases were noted in pY508 and pY531 in the nonreceptor hematopoietic tyrosine kinases Lyn and Fyn at both the 16-hour and 3-day time points compared with those in non- $\alpha 6$ -deleted ALL cells (Figure 5B). Lyn and Fyn are approximately 60 kDa, and these tyrosine residues are significant in that their phosphorylation inhibits the activity of these tyrosine kinases.⁴¹⁻⁴³ Thus, kinases with increased phosphorylation of these residues have reduced tyrosine kinase activity. To further address the significance of Lyn and Fyn, $\alpha 6$ wild-type (nontamoxifen-treated ERT²-Cre $\alpha 6^{fl/fl}$) B-ALL cells were treated with siRNA against Lyn and Fyn. Serum starvation of $\alpha 6$ wild-type murine ALL cells, which makes such cells quiescent, reduced the levels of the phosphorylation of Y418 of Src and increased those of Y507 of Lyn and Y530 of Fyn, which is consistent with downregulation of Src and Lyn tyrosine kinase activity (supplemental Figure 13A). Stimulation of the cells with serum reversed this downregulation, resulting in a decrease in Lyn pY507 and an increase in Src pY418. Interestingly, the activation of both kinases was reduced when cells represented a mixed population with clones that carry $\alpha 6$ deletions and others without deletion using a short-term treatment (16 hours to avoid increasing cell death) with tamoxifen.

We also examined the connection between the Src family kinases and $\alpha 6$ in a different setting using human ALL cells cocultured with OP9 cells as a stimulus and the $\alpha 6$ function-blocking mAb P5G10 (supplemental Figure 13B-C). In this system, plating ALL cells on OP9 cells caused an increase in the levels of total Src protein, but cells treated with P5G10 had

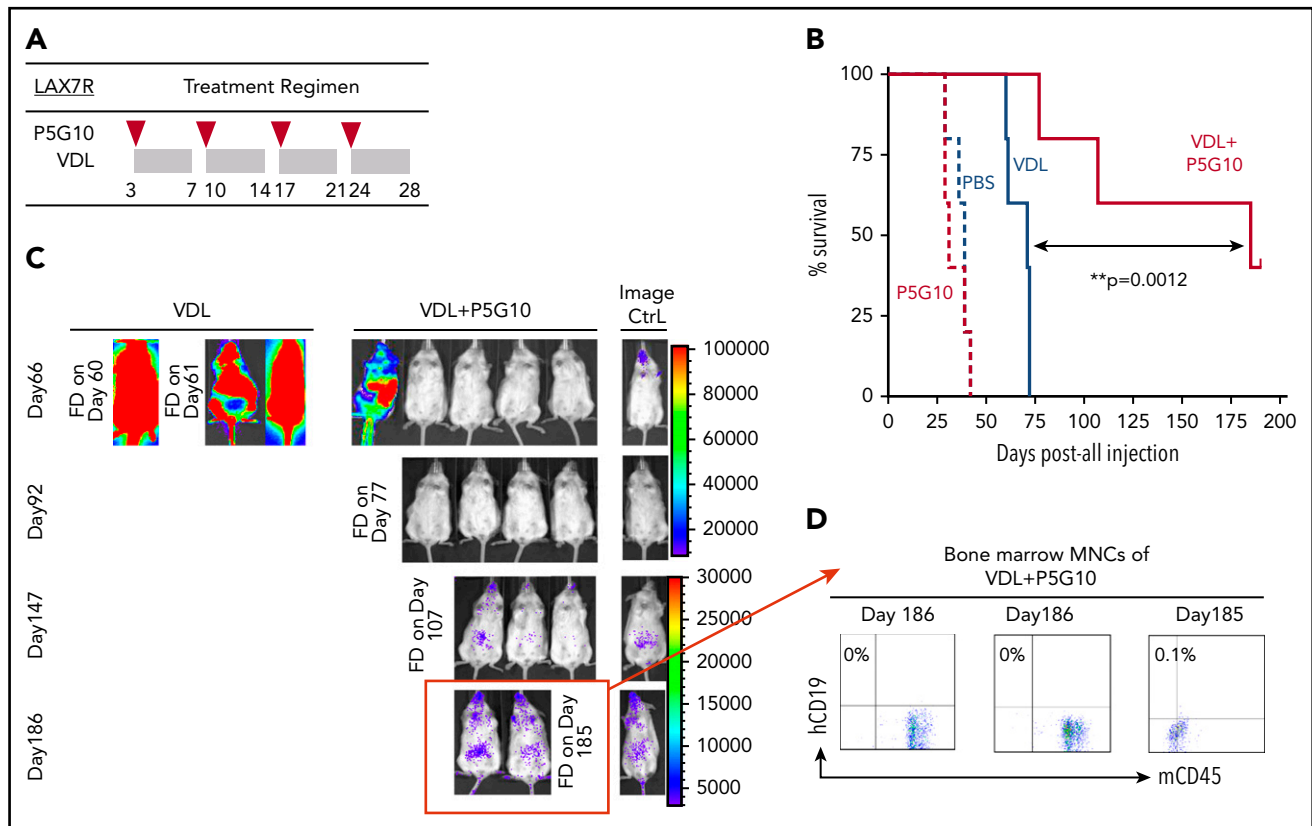


Figure 3. $\alpha 6$ blockade sensitizes leukemia cells to chemotherapy in vivo. (A) The treatment regimen used in NSG mice injected with primary B-ALL cells (LAX7R) consisted of the intraperitoneal administration of PBS ($n = 5$); an $\alpha 6$ -blocking Ab (P5G10) ($n = 5$) (administered intraperitoneally on days 3, 10, 17, and 24 after leukemia injection as indicated by the red triangles); vincristine (10.5 mg/kg), dexamethasone (8 mg/kg), and L-asparaginase (800 IU/kg) (VDL) ($n = 5$) (as indicated by the gray rectangles); or VDL + P5G10 ($n = 5$). (B) The Kaplan-Meier survival curve was analyzed, and the MST was calculated for each group: PBS (MST = 39 days), P5G10 (MST = 31 days), VDL (MST = 71 days), VDL + P5G10 (MST = 185 days). (C) Bioluminescence imaging of mice on days 66, 92, 147, and 186 after leukemia cell transfer. A mouse with no leukemia cell injection treated only with luciferin at the time of imaging was included as a background control (Image CtrlL) (right panel). One experiment is shown. (D) Flow cytometric analysis of human (h) CD19 and murine (m) CD45 in BM mononuclear cells (MNCs) from 3 animals euthanized on day 186 after injection or found dead (FD) on day 185 after injection.

reduced Src levels. Taken together, these results in human and murine B-ALL cells implicate Src family kinases in the decrease in cell viability that accompanies $\alpha 6$ deletion. In addition, we tested whether inhibition of Csk using siRNA rescued the apoptosis induced by P5G10 (supplemental Figure 14A-B). LAX7R cells were transfected with CSK siRNA and treated with either P5G10 or an IgG1 control 1 hour later. After 3 days, the ALL cells were harvested and analyzed by flow cytometry to measure apoptosis, and the protein lysates were analyzed by western blotting. Interestingly, P5G10 induced apoptosis in the cells treated with scrambled control, but there was no P5G10-associated induction of apoptosis after Csk siRNA treatment. This indicates that inhibition of Csk rescued the P5G10 induced apoptosis.

To test whether Src inhibitors can phenocopy the apoptotic effects of P5G10, primary B-ALL cells (ICN24) were treated with different doses of the 3 different Src inhibitors⁴⁴⁻⁴⁶ for 4 days after plating on OP9 cells (supplemental Figure 15). Cell viability was not affected (supplemental Figure 15A), but all 3 tested Src inhibitors decreased proliferation, as illustrated by the GR50 values (growth rate is reduced by half)⁴⁷ determined on the basis of the inhibition of 50% of the proliferation of ICN24 cells (supplemental Figure 15 B-C) and the decrease in Src levels (supplemental Figure 15D). Thus, Src inhibitors do not induce

apoptosis in a manner similar to that of P5G10 but they do reduce $\alpha 6$ protein expression.

$\alpha 6$ deletion sensitizes murine leukemia cells to tyrosine kinase inhibition

To determine the role of integrin $\alpha 6$ in chemotherapy resistance, BCR-ABL-1⁺ $\alpha 6$ ^{fl/fl} EmptyER^{T2} or CreER^{T2} cells were treated with tamoxifen to induce $\alpha 6$ deletion simultaneously with nilotinib treatment (0.02 and 0.2 μ M) or the dissolvent dimethyl sulfoxide (0.1%) as a control. Deletion of $\alpha 6$ (Figure 6A; supplemental Figure 16) markedly decreased the resistance of CreER^{T2} cells to nilotinib compared with the resistance of EmptyER^{T2} cells, as demonstrated by the percentage of apoptotic leukemia cells on day 5 after treatment with nilotinib (0.02 μ M) (94.9% \pm 1.1% vs 70.3% \pm 1.7%; $P < .05$) (Figure 6B), indicating that $\alpha 6$ deletion sensitizes murine leukemia cells to tyrosine kinase inhibition.

Combining $\alpha 6$ deletion with tyrosine kinase inhibition eradicates murine leukemia

To determine the effect of $\alpha 6$ deletion on leukemia progression in vivo, $\alpha 6$ -competent $\alpha 6$ ^{fl/fl} BCR/ABL1⁺p210 CreER^{T2+} or EmptyER^{T2} cells were lentivirally labeled with luciferase and transferred into NSG mice. After 3 days, $\alpha 6$ deletion was induced by administering tamoxifen to all animals with 2 cycles of 5 days each.

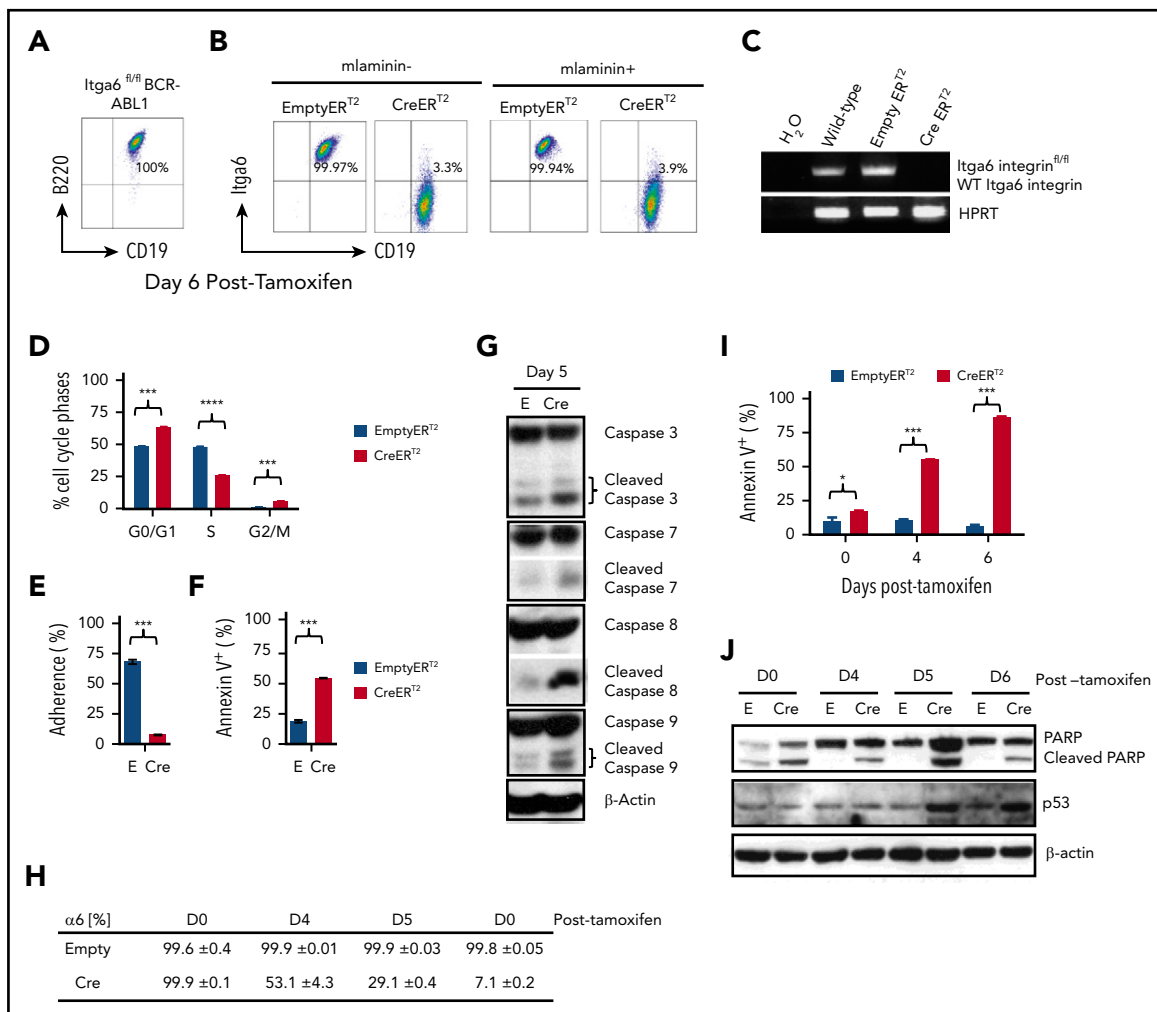


Figure 4. Integrin $\alpha 6$ deletion induces the death and apoptosis of murine BCR-ABL1⁺ (p210) ALL cells. (A) Immunophenotype of murine BCR-ABL1 (p210)⁺ ALL cells. (B) Deletion of $\alpha 6$ induced by tamoxifen (1.5 μ M) was confirmed by flow cytometry 6 days after tamoxifen treatment. Left panel: cells not plated on mlaminin-1; right panel: cells plated on mlaminin+. (C) Deletion of $\alpha 6$ was confirmed by genomic PCR. Hypoxanthine-guanine phosphoribosyltransferase (HPRT) was used as a PCR DNA and loading control. (D) Cell cycle analysis of CreER^{T2} and EmptyER^{T2} cells on day 5 after deletion by bromodeoxyuridine flow cytometry. *** P < .001 and **** P < .0001 by 1-way ANOVA with post hoc analysis (Tukey test). (E) Percentage of adherent CreER^{T2} and EmptyER^{T2} cells on mlaminin-coated plates on day 5. *** P < .001 by Student t test. (F) Apoptosis analysis by annexin V and 7-AAD staining of CreER^{T2} and EmptyER^{T2} cells at day 5 after deletion. *** P < .001 by Student t test. (G) Western blot of cleaved caspases 3, 7, 8, and 9 in CreER^{T2} and EmptyER^{T2} cell lysates on day 5 after deletion. β -actin, loading control. (H) Percentage of $\alpha 6$ and (I) annexin V expression in CreER^{T2} and EmptyER^{T2} cells at various time points after $\alpha 6$ deletion, as determined by flow cytometry. Mean \pm standard deviation is shown. * P < .05 and *** P < .001 by 1-way ANOVA with post hoc analysis (Tukey test). (J) Western blot of cleaved poly (ADP-ribose) polymerase (PARP) and p53 in CreER^{T2} and EmptyER^{T2} cells on various days (days 0, 4, 5, and 6) after tamoxifen-induced $\alpha 6$ deletion. β -actin, loading control. One of 3 experiments is shown.

Bioluminescence imaging showed a significant delay in the progression of $\alpha 6$ -deleted leukemia cells compared with that of $\alpha 6$ -competent cells (Figure 6C, saline-treated animals) with an MST of 27 days compared with 54.5 days after leukemia cell injection (log-rank test $P = .0021$). Moreover, in vivo deletion of $\alpha 6$ in combination with nilotinib significantly prolonged survival after leukemia cell transfer, because recipients of $\alpha 6$ -competent cells died of leukemia with an MST of 39.5 days despite nilotinib treatment, whereas nilotinib-treated recipients of $\alpha 6$ -ablated leukemia cells survived ($P = .0018$) (Figure 6D). The deletion status of $\alpha 6$ was determined when the animals were euthanized. This analysis confirmed that $\alpha 6$ had been deleted in most cells (Figure 6E-F). Animals were euthanized on day 127 after leukemia injection for the analysis of MRD. We found no detectable BCR-ABL1 mRNA expression by flow cytometry (Figure 6G) or BCR-ABL1 representation in genomic DNA by PCR (Figure 6H) in splenic cells and BM cells. Similar results were obtained in a second

experiment, in which in vivo deletion of $\alpha 6$ was induced on day 6 after leukemia injection to account for possible effects on engraftment contributing to the survival benefit (supplemental Figure 17).

Discussion

Our study identified $\alpha 6$ as a marker of HSCs,¹⁶ and its expression was also recently reported in B-ALL cells.²⁰ Our results clearly show that all the cells in the B-ALL populations tested, including those with different genetic lesions such as BCR/ABL1 fusions, KRAS^{G12V}, and MLL rearrangements, express high levels of $\alpha 6$ on their surfaces. On the basis of our analysis of $\alpha 6$ expression in and on normal precursor B cells in healthy human and mouse BM (supplemental Figure 1A-D), such high $\alpha 6$ levels on malignant precursor B cells are abnormal. Because we also found a correlation between increased $\alpha 6$ mRNA levels and the detection of

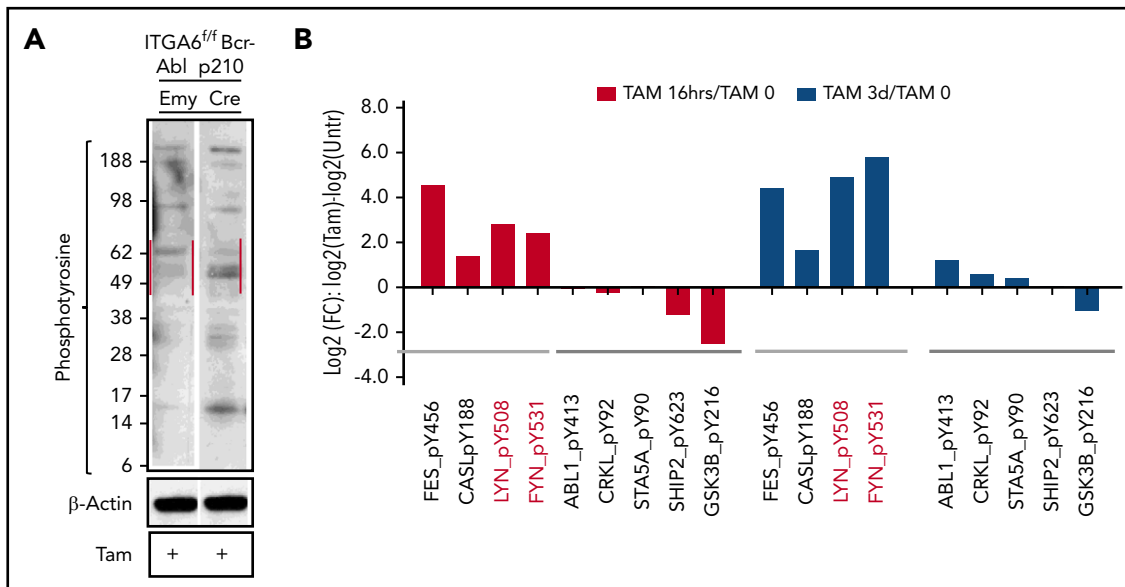


Figure 5. Integrin $\alpha 6$ deletion in murine BCR-ABL1⁺ (p210) B-ALL cells affects Src signaling. (A) $\alpha 6^{f/f}$ BCR-ABL1⁺ (p210) x CreER^{T2} (Cre) and x EmptyER^{T2} (Emy) cells treated with tamoxifen (TAM) for 24 hours followed by western blot analysis using an anti-phosphotyrosine Ab (clone 4G10). One of 3 experiments is shown. (B) Phosphotyrosine proteomics analysis was performed on murine cells treated for 16 hours or 3 days with tamoxifen. The fold changes (FCs) in the selected phosphotyrosine proteins were plotted. One experiment is shown.

MRD after induction chemotherapy in human patient samples, high expression of $\alpha 6$ is likely to provide a benefit to the B-ALL cells that express it. One aspect of this benefit may be the ability to engage with specific elements in protective BM niches. Our results using function-blocking mAbs and human ALL cells demonstrated that B-ALL cells adhere to different laminins via $\alpha 6$. This shows that the integrin is fully functional as an adhesion molecule on such cells, which is highly significant because niches in the BM are rich in laminins.⁴⁸ We were also able to demonstrate that the blocking of $\alpha 6$ with P5G10 impaired adhesion to OP9 BM stromal cells, so it is possible that such stromal cells secrete laminin-rich ECMs to which the ALL cells adhere, although other $\alpha 6$ -mediated contacts may also take place. In addition, ALL cells may contact OP9 cells through other integrins such as integrin $\alpha 4$.^{15,22} Our results demonstrate that $\alpha 6$ -mediated adhesion to laminin or OP9 cells protects different ALL cells from treatment with both VDL conventional chemotherapy and a targeted TKI. This further supports the presence of a link between high $\alpha 6$ expression and MRD persistence. Moreover, it has been reported that $\alpha 6$ -laminin interactions mediate the migration of ALL cells toward the cerebrospinal fluid in vitro.²⁰ Our results suggest that mAbs against $\alpha 6$ could therefore also be useful in preventing CNS relapse.

B-ALL cells can express multiple integrins such as $\alpha 4$ and $\alpha 6$ on the same cell, which suggests that these integrins have distinct functions. One obvious difference is that $\alpha 4$ and $\alpha 6$, as part of $\alpha 4\beta 1$ or $\alpha 6\beta 1$ integrins, engage with distinct extracellular ligands (fibronectin and VCAM-1 vs laminin). Both $\alpha 4$ and $\alpha 6$ have been shown to play distinct roles in homing of normal HSCs.⁴⁹ Differential ECM protein expression may lead to protection of ALL cells via different CAM-DR mechanisms. Here, we show an additional striking difference: the deletion of integrin $\alpha 6$ in murine B-ALL cells causes apoptosis, whereas this does not occur in a similar experimental setup when integrin $\alpha 4$ is deleted (Hsieh et al²²; supplemental Figure 12).

Thus, our experiments show that *Itga6* is much more than a mere adhesive integrin; its loss profoundly affected the phenotype of B-ALL cells and resulted in an accumulation of cells in G₂/M and G₀/G₁ phases, which is consistent with a corresponding increase in p53 levels.⁵⁰ In addition, caspase activation with apoptosis is associated with the long-term loss of $\alpha 6$. The moderate effect of $\alpha 6$ functional inhibition by mAbs on the viability of B-ALL cells adhering to laminin-1 in the presence of normal serum is consistent with the lack of induction of acute caspase cleavage in these cells. However, we found that the expression of the anti-apoptotic IAP family member survivin was induced by chemotherapy treatment and decreased by treatment with P5G10 in LAX7R cells (supplemental Figure 6C), which suggests a specific role for survivin in $\alpha 6$ -mediated survival, which may not be the sole mechanism.

Intracellular signal transduction complexes that are engaged by integrins include Src family kinase (SFK) members, and our experiments provide evidence that the genetic ablation of $\alpha 6$ is correlated with the downregulation of SFK levels and their activation in BCR/ABL1⁺ ALL cells. Consistent with the link between SFK members and $\alpha 6$ function, we found that mice transplanted with BCR/ABL1 kinase-driven leukemia cells, in which $\alpha 6$ was deleted in vivo, had significantly longer survival when treated with nilotinib. There have been several previous reports of links between specific SFK members and B-ALL that demonstrate their effects on BCR-ABL1-like subcategory members (Lyn) as well as Ph⁺ cases, in which they are activated downstream of the BCR-ABL1 kinase. In addition, the role of the SFK Fyn in the progression of chronic myeloid leukemia (CML) to blast crisis has been described.⁵¹ Src family kinases can phosphorylate the BCR-ABL1 SH3-SH2 region and modulate BCR-ABL1-transforming activity.⁵² The Src pathway is also essential for CML cells to survive imatinib treatment and for their transition to lymphoid blast crisis.⁵³ However, although treatment of the Ph⁺ CML cell lines with the Src kinase inhibitors PP2 or A-419259

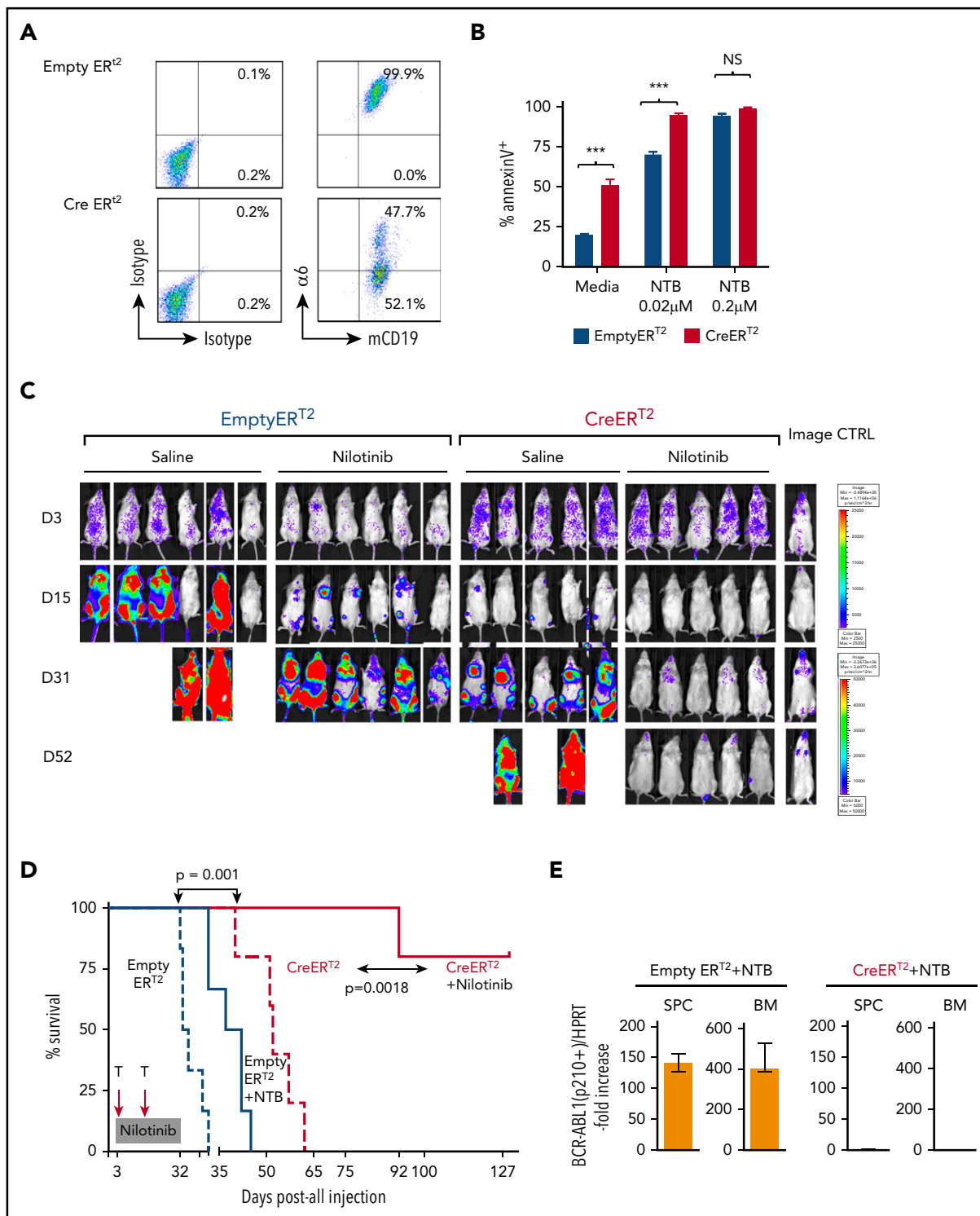


Figure 6. $\alpha 6$ deletion sensitizes murine leukemia cells to tyrosine kinase inhibition in vitro, and the combination of the in vivo deletion of $\alpha 6$ with tyrosine kinase inhibition eradicates leukemia cells. $\alpha 6^{fl/fl}$ BCR-ABL1⁺ (p210) CreER^{T2} and EmptyER^{T2} cells were plated onto tissue culture plates (without further coating) and treated with tamoxifen (1.5 μ M) and nilotinib (0.02 μ M or 0.2 μ M) for 5 days. (A) Deletion of $\alpha 6$ was determined by flow cytometry. (B) Cell viability was determined by annexin V detection using flow cytometry. The y-axis indicates the percentage of annexin V-positive cells. Mean \pm standard deviation is shown. *** $P < .001$. (C) Bioluminescence imaging of mice injected with luciferase-labeled murine $\alpha 6^{fl/fl}$ BCR-ABL1⁺ CreER^{T2} and EmptyER^{T2} ALL cells followed by treatment with tamoxifen to delete $\alpha 6$ with or without nilotinib on the indicated days after ALL cell injection. One in vivo experiment is shown. (D) Kaplan-Meier survival analysis for the MST determination in each group: EmptyER^{T2} (n = 6), MST = 27 days; CreER^{T2} (n = 5), MST = 54.5 days; EmptyER^{T2} + nilotinib (n = 6), MST = 39.5 days; CreER^{T2} + nilotinib (n = 5), MST = undefined because 4 of 5 mice remained alive until the end of follow-up. * $P = .0001$, log-rank test. (E) Detection of murine BCR-ABL1 (p210⁺) cells in spleen cells (SPCs) or BM by qRT-PCR. Error bars are from the 2 comparison groups (Empty ER^{T2} + NTB vs CRE ER^{T2} + NTB). (F) $\alpha 6$ deletion was confirmed by flow cytometry in BM cells from leukemic mice injected with $\alpha 6^{fl/fl}$ BCR-ABL1⁺ CreER^{T2} or EmptyER^{T2} cells treated with nilotinib. Donor white blood cells (WBCs) were labeled with CD45.2⁺, and recipient WBCs were CD45.1⁺. (H) Genomic PCR of BCR-ABL1 was performed on cells isolated from the spleen and BM of mice treated with EmptyER^{T2} + nilotinib (NTB) and CreER^{T2} + NTB. Murine HPRT (mHPRT) was used as an internal PCR DNA control.

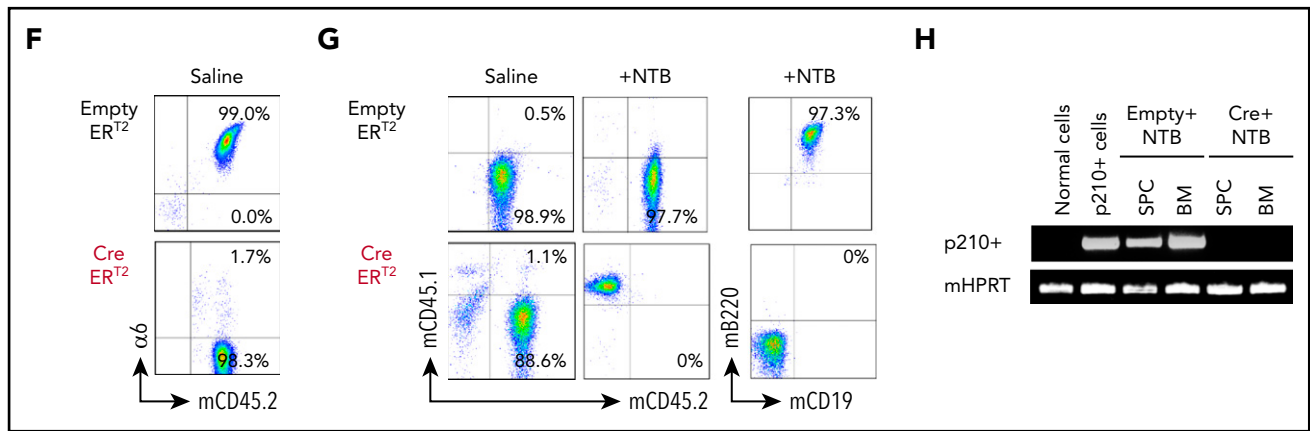


Figure 6. (Continued).

resulted in growth arrest and the induction of apoptosis,⁵⁴ our data show that Src inhibitors only decrease the growth of primary B-ALL cells and do not affect their viability (supplemental Figure 15). Thus, Src inhibitors have a less pronounced effect on B-ALL than P5G10, suggesting that the signal transduction processes and protein complexes assembled intracellularly after Itga6 engagement are only minimally dependent on SFK members.

Apart from its utility as a clinical risk factor indicator, the high levels of $\alpha 6$ expression on Ph⁺ ALL blasts found in our study suggest that it could be used as a therapeutic target. It is important to note that this integrin is also expressed at low levels on hematopoietic cells, as shown in our studies. It is also expressed on other cell types, including epithelial cells. The absence of $\alpha 6$ in the intestinal epithelium induced the development of inflammation-associated colorectal cancers in mice.⁵⁵ Because these mice display epithelial detachment from the basement membrane, systemic anti- $\alpha 6$ therapy could have deleterious effects on the intestine. However, as we have shown here, complete gene ablation (total loss of Itga6 protein) has a much more drastic effect than the inhibition of the engagement of the Itga6 extracellular domain with its ligand laminin through function-blocking Abs. Indeed, we have no evidence that such Ab treatment has any deleterious effects on normal tissues in mouse models (data not shown).

We have previously reported that Itga4 is targetable by an existing US Food and Drug Administration–approved drug (natalizumab).²² Clearly, the function of $\alpha 6$ differs from that of $\alpha 4$, suggesting that efforts to target $\alpha 6$ in B-ALL may be warranted even though $\alpha 4$ -directed agents already exist. In summary, we demonstrated that targeting $\alpha 6$ by itself induces apoptosis, and $\alpha 6$ ablation (pharmacologic and genetic) sensitizes ALL cells to nilotinib or chemotherapy, suggesting that the targeting of the integrin $\alpha 6$ may serve as a method for treating refractory ALL.

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Authorship

Contribution: Y.-M.K. was the principal investigator and takes primary responsibility for the article; E.J.G., H.N.K., Y.-T.H., Y.R., H.A.O., S.L., J.P., E.P., and L.K. performed the laboratory work and analyzed the data for this study; H.G. performed the bioinformatics analysis of the MRD data; C.L.W. and W.L.C. provided the MRD data; A.D.A., E.G.-L., H.B., N.H., M.M., and E.W. provided antibodies, reagents, $\alpha 6^{fl/fl}$ line, and critical expertise and wrote the manuscript; T.G.G., A.M., and J.t.H. performed the proteomics study and analysis; M.J.O. analyzed and presented the histopathologic data; E.O. and S.D.M. provided histologic samples; D.B., C.P., H.A.-A., and A.S.W. provided patient samples and critical expertise and wrote the manuscript; and E.J.G., H.N.K., Y.-T.H., Y.R., H.B., N.H., and Y.-M.K. wrote the manuscript.

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Elisabeth Georges-Labouesse died in 2012.

ORCID profiles: E.P., 0000-0001-8432-5168; S.D.M., 0000-0003-2867-1298; E.O., 0000-0002-1487-6818; M.J.O., 0000-0001-6419-2513; A.D.A., 0000-0003-1114-8441; A.M., 0000-0002-0063-2513; T.G.G., 0000-0001-8574-9181; N.H., 0000-0003-2769-2273; Y.-M.K., 0000-0003-3793-6438.

Correspondence: Yong-Mi Kim, Division of Hematology-Oncology, Children’s Hospital Los Angeles, University of Southern California, 4650 Sunset Blvd, Mailstop #57, Los Angeles, CA 90027; e-mail: ymkim@chla.usc.edu.

Footnotes

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For original data, please contact Yong-Mi Kim at ymkim@chla.usc.edu.

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