Reprogramming of primary human Philadelphia chromosome-positive B cell acute lymphoblastic leukemia cells into nonleukemic macrophages

James Scott McClellan[a,1], Christopher Dove[a,1], Andrew J. Gentles[b], Christine E. Ryan[a], and Ravindra Majeti[a,2]

Departments of [a]Medicine, Division of Hematology, Cancer Institute, and Institute for Stem Cell Biology and Regenerative Medicine, and [b]Radiology, Stanford University School of Medicine, Stanford, CA 94305

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BCR–ABL1$^+$ precursor B-cell acute lymphoblastic leukemia (BCR–ABL1$^+$ B-ALL) is an aggressive hematopoietic neoplasm characterized by a block in differentiation due in part to the somatic loss of transcription factors required for B-cell development. We hypothesized that overcoming this differentiation block by forcing cells to reprogram to the myeloid lineage would reduce the leukemogenicity of these cells. We found that primary human BCR–ABL1$^+$ B-ALL cells could be induced to reprogram into macrophage-like cells by exposure to myeloid differentiation-promoting cytokines in vitro or by transient expression of the myeloid transcription factor C/EBPα or PU.1. The resultant cells were clonally related to the primary leukemic blasts but resembled normal macrophages in appearance, immunophenotype, gene expression, and function. Most importantly, these macrophage-like cells were unable to establish disease in xenograft hosts, indicating that lineage reprogramming eliminates the leukemogenicity of BCR–ABL1$^+$ B-ALL cells, and suggesting a previously unidentified therapeutic strategy for this disease. Finally, we determined that myeloid reprogramming may occur to some degree in human patients by identifying primary CD14$^+$ monocytes/macrophages in BCR–ABL1$^+$ B-ALL patient samples that possess the BCR–ABL1$^+$ translocation and clonally recombined VDJ regions.

acute lymphoblastic leukemia | lineage reprogramming | transdifferentiation | macrophage | CCAAT/enhancer binding protein alpha

Precursor B-cell acute lymphoblastic leukemia (B-ALL) is an aggressive hematopoietic neoplasm characterized by recurrent chromosomal abnormalities. One poor risk subtype of B-ALL is characterized by the presence of the Philadelphia chromosome (Ph), formed by the *t*(9, 22) (q34;q11.2) translocation, which creates the fusion protein BCR–ABL1, a constitutively active tyrosine kinase. BCR–ABL1$^+$ B-ALL is the most common molecular subtype of ALL in adults, comprising ∼20% of cases (1). Despite the addition of tyrosine kinase inhibitors to treatment regimens for BCR–ABL1$^+$ B-ALL, prognosis for adults with this disease remains poor, highlighting the need for improved treatments (2).

Recent studies using single nucleotide polymorphism (SNP) arrays have shown that BCR–ABL1$^+$ B-ALL is characterized by frequent deletions of key lymphoid transcription factors. For example, *IKZF1*, which encodes the transcription factor Ikaros, is deleted in >80% of BCR–ABL1$^+$ B-ALL cases, and PAX5, a key transcription factor in B-cell development, is deleted in >50% of BCR–ABL1$^+$ B-ALL cases (3, 4). Murine models have shown that loss of PAX5 and partial loss of Ikaros both lead to B-cell maturation arrest at the pro–B-cell stage (5, 6). The high frequency of loss of these genes in BCR–ABL1$^+$ B-ALL suggests that B-cell maturation arrest is critical to the pathogenesis of this disease.

Interestingly, although unable to form mature B cells, both PAX5-deficient and Ikaros-deficient pro-B cells retain the capacity to lineage reprogram (sometimes referred to as "transdifferentiation," henceforth "reprogram") into cells of the myeloid lineage, specifically macrophages, upon exposure to macrophage-colony stimulating factor (M-CSF) (5, 7). Similarly, several studies have shown that both wild-type common lymphoid progenitors and B-cell precursors can be manipulated to reprogram into macrophages by enforced expression of bZip family transcription factor CCAAT/enhancer binding protein alpha (C/EBPα) (8, 9). More recently, Rapino et al. have shown that a subset of human B-ALL cell lines can be induced to reprogram to the myeloid lineage by forced expression of C/EBPα (10). These findings raise the possibility that primary human BCR–ABL1$^+$ B-ALL blasts can be reprogrammed in a similar manner.

The concept of forcing malignant cells to terminally differentiate as a therapeutic strategy was proposed several decades ago (11). To date, however, differentiation therapy has only been used routinely in a subtype of acute myeloid leukemia, namely, acute promyelocytic leukemia (APL). Currently, >95% of patients with APL can expect to be cured by differentiation therapy alone without the use of cytotoxic chemotherapy (12). In contrast, no differentiating therapies have proven effective in the treatment of B-ALL. Normal B-cell development requires successful passage through multiple developmental checkpoints (e.g., the expression of a functional pre–B-cell receptor) involving coordinated expression of multiple gene programs (13). Given these requirements, forcing malignant B-ALL cells to differentiate into mature, naïve B cells may not be possible. However, similar to *IKZF1*$^{-/-}$ and *PAX5*$^{-/-}$ pro-B cells, we reasoned that primary human B-ALL cells might be poised to reprogram to the myeloid lineage.

Significance

Precursor B cell acute lymphoblastic leukemia (B-ALL) is an aggressive cancer of white blood cells with a poor prognosis. The cancerous cells in this disease are immature B cells, which are unable to fully differentiate into normal B cells. We show here that cancerous cells from B-ALL patients can be reprogrammed, causing them to change into cells that resemble normal macrophages and can perform macrophage-associated functions such as the consumption of bacteria. Importantly, unlike typical B-ALL cells, these reprogrammed cells are no longer able to cause disease in immunodeficient mice. Finally, we show that this reprogramming process may occur to some degree in patients with B-ALL. This indicates that reprogramming B-ALL cells into macrophages might represent a previously unidentified therapeutic strategy.

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[1]J.S.M. and C.D. contributed equally to this work.

[2]To whom correspondence should be addressed. Email: rmajeti@stanford.edu.

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We chose to focus our analysis on BCR–ABL1$^+$ cases of B-ALL both because this subtype of B-ALL has a high frequency of *PAX5* and *IKZF1* deletions and because BCR–ABL1 positivity is associated with a particularly poor prognosis. Furthermore, expression of receptors for myeloid cytokines by B-ALL cells has been described (14). Because myeloid reprogramming of *PAX5*$^{-/-}$ and *IKZF1*$^{-/-}$ pro-B cells can be triggered by exposure to myeloid cytokines (5, 7), we reasoned that exposure to analogous human cytokines might similarly induce myeloid reprogramming in primary human BCR–ABL1$^+$ B-ALL cells. We report here that BCR–ABL1$^+$ B-ALL cells can indeed be forced to reprogram, and the resultant cells are phenotypically and functionally similar to normal human macrophages.

Results

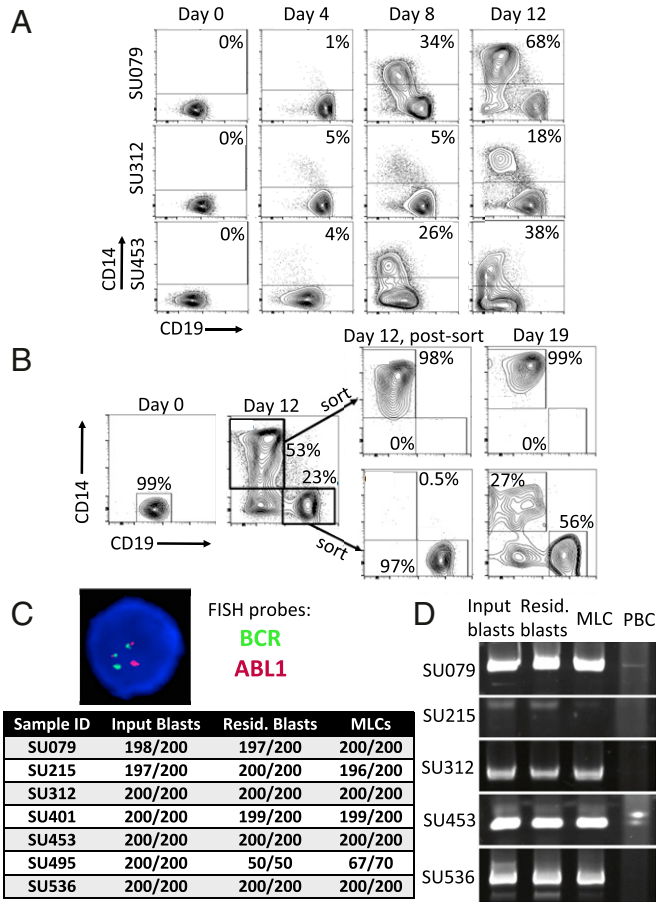
BCR–ABL1$^+$ B-ALL Blasts Reprogram into CD14^{hi}/CD19^{lo} Macrophage-Like Cells When Cultured in the Presence of Myeloid Differentiation-Promoting Cytokines. To investigate whether primary human BCR–ABL1$^+$ B-ALL blasts could be induced to reprogram to the myeloid linage upon exposure to myeloid differentiation-promoting cytokines, we first sought to isolate a pure population of leukemic blasts, thereby excluding rare contaminating non-malignant hematopoietic stem and myeloid progenitor cells. We isolated the CD19$^+$CD34$^+$ blasts to greater than 98% purity by FACS from 12 adult BCR–ABL1$^+$ B-ALL cases ("input blasts") (Fig. 1*A* and *SI Appendix*, Fig. S1 *A–L*) and cultured them with the myeloid cytokines interleukin 3 (IL-3), M-CSF, and granulocyte/monocyte (GM)-CSF. We additionally added Fms-related tyrosine kinase 3 ligand (FLT3L) and interleukin 7 (IL-7) to these cultures, as we found that these cytokines improved in vitro survival of B-ALL blasts, although having minimal reprogramming capacity in the absence of the myeloid cytokines (*SI Appendix*, Fig. S2). The clinical characteristics of the 12 patients examined in this study are detailed in *SI Appendix*, Table S1.

The input blasts were cultured under these conditions for 12 d, during which we used flow cytometry to monitor for the up-regulation of the macrophage marker CD14 at several time points. In 7 of 12 cases, >10% of the cells expressed CD14 after exposure to myeloid cytokines for 12 d, suggesting that, in a majority of adult BCR–ABL1$^+$ cases, B-ALL blasts are capable of reprogramming into macrophage-like cells (MLCs) (Fig. 1*A* and *SI Appendix*, Fig. S1 *A–L*).

We additionally tested the reprogramming potential of five BCR–ABL1$^-$ cases and found that B-ALL blasts in two of five cases were capable of reprogramming into MLCs (*SI Appendix*, Fig. S1 *M–Q* and Table S2), indicating that reprogramming capacity is not limited to BCR–ABL1$^+$ cases.

Further investigation of the molecular genetics of these BCR–ABL1$^+$ B-ALL cases did not identify another cytogenetic abnormality or deletion of a particular transcription factor (e.g., *IKZF1* loss) associated with myeloid reprogramming capacity (*SI Appendix*, Table S1 and Figs. S3 and S4). Gene set enrichment analysis (GSEA) (15, 16) of FACS-purified blasts from 10 BCR–ABL1$^+$ cases, 5 that underwent reprogramming and 5 that did not, found that genes associated with mitosis and cell cycle were significantly up-regulated in those cases that underwent myeloid reprogramming (*SI Appendix*, Fig. S5), suggesting that high mitotic activity correlates with myeloid reprogramming in vitro.

On days 4, 8, and 12 of culture, we noted residual CD19^{high} cells ("residual blasts") that were phenotypically similar to input blasts despite continuous exposure to myeloid cytokines (Fig. 1*A*). To examine whether this population was refractory to reprogramming by cytokines, this population was purified by FACS and recultured under the same conditions for an additional 7 d. Subsequent analysis indicated that these residual blasts retained the potential to reprogram into MLCs (Fig. 1*B*). We additionally FACS purified MLCs from our B-ALL cultures and recultured them in the presence of myeloid cytokines. Flow cytometry analysis of these cultures after an additional 7 d demonstrated that the CD14^{high} phenotype was stable, as we did not observe any evidence of reversion to a CD19^{high} blast-like phenotype (Fig. 1*B* and *SI Appendix*, Fig. S6).



Sample ID	Input Blasts	Resid. Blasts	MLCs
SU079	198/200	197/200	200/200
SU215	197/200	200/200	196/200
SU312	200/200	200/200	200/200
SU401	200/200	199/200	199/200
SU453	200/200	200/200	200/200
SU495	200/200	50/50	67/70
SU536	200/200	200/200	200/200

Fig. 1. BCR–ABL1$^+$ B-ALL blasts reprogram into CD14^{high}/CD19^{low} macrophage-like cells when cultured in the presence of myeloid differentiation-promoting cytokines. (*A*) CD19$^+$/CD34$^+$ B-ALL blasts were isolated by FACS and cultured with myeloid cytokines in vitro for 12 d. CD19 and CD14 expression was determined by flow cytometry on the indicated days. The three patient samples shown are representative of seven adult BCR–ABL1$^+$ B-ALL cases (see also *SI Appendix*, Fig. S1). (*B*) At day 12, CD14^{high} and CD19^{high} cells were isolated by FACS and placed back in culture with myeloid cytokines, followed by repeat analysis at day 19. Data are representative of three BCR–ABL1$^+$ B-ALL cases (see also *SI Appendix*, Fig. S6). (*C*) FISH staining demonstrating the presence of the BCR–ABL1 *t*(9, 22) translocation in a FACS-purified CD14^{high} cell. Green, BCR probe; red, ABL1 probe. FISH analysis was quantified for all seven patient samples; the fraction of cells positive for BCR–ABL1 is shown in the *Inset* table. (*D*) VDJ spectrotyping demonstrating a monoclonal banding pattern in input blasts, residual blasts, and B-ALL–derived macrophage-like cells (MLCs) in five individual B-ALL cases. The fourth lane contains a polyclonal B-cell DNA control (PBC). The gel pictures are cropped to show only the relevant size range for the primer set used.

To further demonstrate that the in vitro derived MLCs originate from clonal B-ALL blasts and not rare contaminating normal cells, we FACS purified MLCs from our cultures and subjected them to fluorescence in situ hybridization (FISH) for the presence of the BCR–ABL1 translocation. In all seven of the samples that demonstrated robust reprogramming, ≥98% of the MLCs scored positive for the BCR–ABL1 translocation, suggesting that these cells were derived from the input blasts (Fig. 1*C*). Additionally, SNP analysis from three samples demonstrated that MLCs possess the same copy number variations (CNVs) as the residual blasts (*SI Appendix*, Fig. S7). For samples

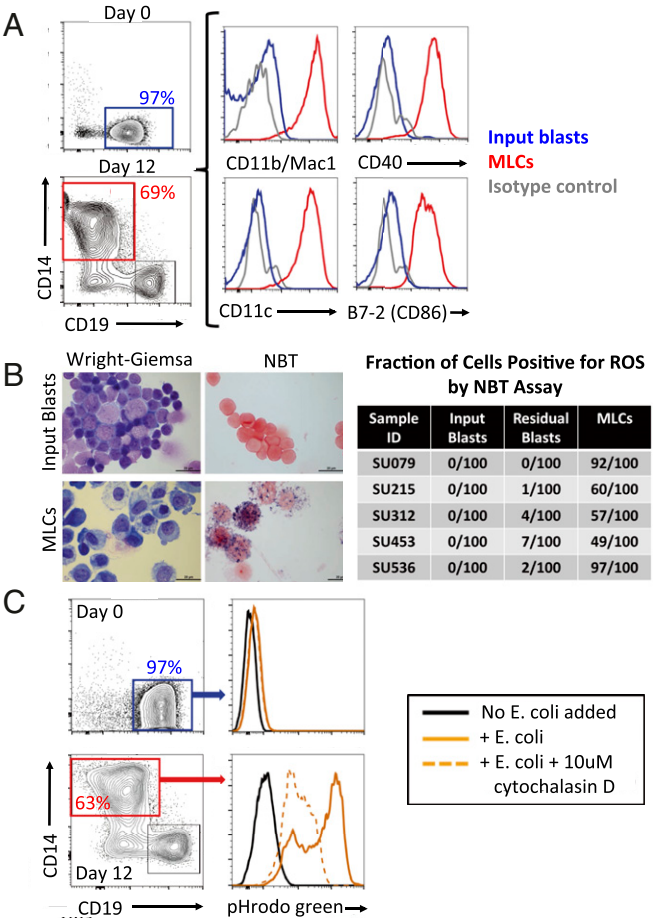
SU079 and SU453, the CNV pattern between input blasts and MLCs was the same as well.

We considered the possibility that MLCs are derived from rare BCR–ABL1$^+$ preleukemic precursors rather than leukemic B-ALL blasts. We took advantage of the fact that the Ig heavy chain (IgH) locus is rearranged in >90% of adult B-ALL cases, providing a leukemia-specific genetic marker in addition to the BCR–ABL1 translocation (17). Using FACS, we isolated input blasts, residual blasts, and MLCs after 12 d of culture and performed mutliplex PCR to detect clonal IgH rearrangements in genomic DNA using primers validated in the BIOMED-2 study (18). In five of six BCR–ABL1$^+$ B-ALL samples tested, we detected an identical clonal IgH rearrangment in the input blasts, residual blasts, and MLCs (Fig. 1*D*). In the remaining sample (SU401), no monoclonal banding pattern was detected in any cell population, likely indicating that this sample had a germ-line (nonrearranged) IgH locus. Thus, we determined that primary human BCR–ABL1$^+$ B-ALL blasts can undergo myeloid reprogramming when cultured in vitro with myeloid differentiation-promoting cytokines.

B-ALL–Derived MLCs Express Cell Surface Markers and Exhibit Functional Properties Typical of Normal Macrophages. B-ALL–derived MLCs were identified based on expression of the monocyte/macrophage-specific cell surface marker CD14. To further characterize MLCs, we used flow cytometry to analyze the expression of additional cell surface markers known to be highly expressed on normal macrophages. Relative to input blasts, MLCs expressed high levels of CD11b/Mac1, CD11c, and CD40L (Fig. 2*A* and *SI Appendix*, Fig. S8). Furthermore, these cells up-regulated the costimulatory molecule CD86/B7-2 (Fig. 2*A* and *SI Appendix*, Fig. S8), suggesting that they may be capable of providing costimulation to B-ALL–reactive autologous T cells. Finally, MLCs exhibited characteristic large, foamy cell morphology typical of normal macrophages (Fig. 2*B*).

We next sought to determine whether MLCs were functionally similar to normal macrophages that generate reactive oxygen species (ROS) to kill pathogens. This ability to generate ROS can be assessed using the nitroblue tetrazolium (NBT) reduction assay that results in the formation of a black precipitate through the reaction of NBT with ROS. Using this assay, we determined that MLCs, but not input blasts or residual blasts, effectively generated ROS (Fig. 2*B*). In addition to generation of ROS, phagocytosis is a functional property of normal macrophages. To test whether MLCs also possess phagocytic capacity, we determined their ability to phagocytose *Escherichia coli* bacteria. FACS-purified BCR–ABL1$^+$ B-ALL blasts from four patients were cultured with myeloid cytokines for 12 d and then incubated with *E. coli* labeled with a dye (pHrodo Green) that fluoresces only at a low pH, as would be encountered upon phagocytosis in phagosomes and lysosomes. Unlike primary and residual B-ALL blasts, MLCs readily phagocytosed pHrodo Green-labeled *E. coli*, and this uptake was partially inhibited by cytochalasin D, an inhibitor of phagocytosis (Fig. 2*C* and *SI Appendix*, Fig. S9). Thus, CD14^{high} MLCs, but not CD19^{high} input or residual blasts, possess true phagocytic function typical of normal macrophages.

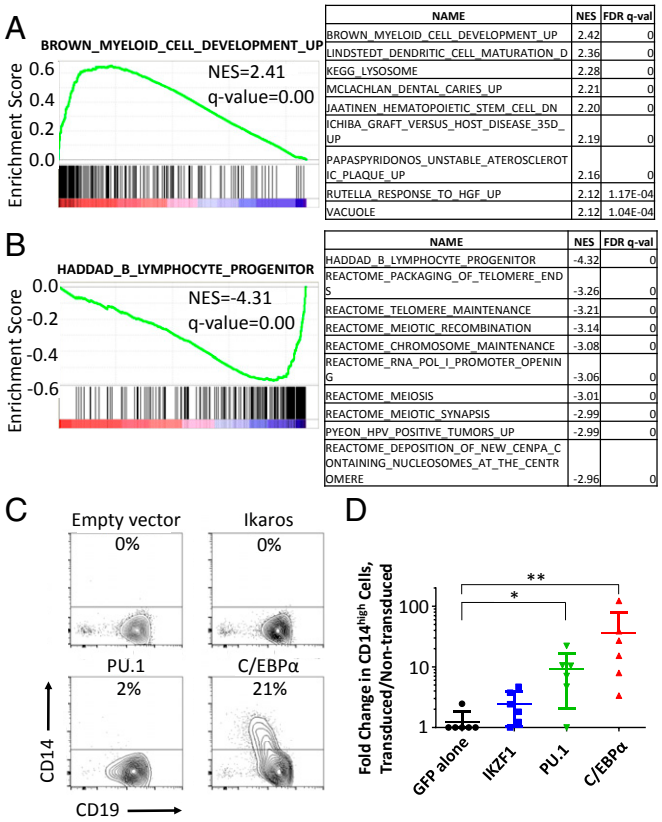
Gene Expression Profiling of MLCs Demonstrates Down-Regulation of an Immature B-Cell Signature and Up-Regulation of a Myeloid Gene Signature. We next sought to investigate the mechanism by which myeloid reprogramming of B-ALL blasts into MLCs occurs through analysis of global gene expression changes. We generated microarray data from FACS-purified matched input blasts and MLCs from five patient samples and identified 3,143 genes that were at least fourfold more highly expressed in MLCs and 228 genes that were fourfold more highly expressed in input blasts [false discovery rate (FDR) <10% for all genes]. GSEA demonstrated that, relative to input blasts, MLCs exhibited up-regulation of myeloid lineage-associated genes and down-regulation



Sample ID	Input Blasts	Residual Blasts	MLCs
SU079	0/100	0/100	92/100
SU215	0/100	1/100	60/100
SU312	0/100	4/100	57/100
SU453	0/100	7/100	49/100
SU536	0/100	2/100	97/100

Fig. 2. B-ALL–derived MLCs express cell surface markers and exhibit functional properties typical of normal macrophages. (*A*) Cell surface expression of CD11b, CD11c, CD40, and B7-2 (CD86) on CD19^{high} B-ALL blasts (input blasts, blue) and day 12 MLCs (red). Shown is flow cytometric analysis of a B-ALL case that is representative of four separate cases (*SI Appendix*, Fig. S8). (*B*) Wright–Giemsa staining (*Left*) and nitroblue tetrazolium (NBT, *Right*) staining of FACS-purified input blasts and MLCs. Images shown are representative of six individual cases. The fraction of cells in each group positive for ROS generation is shown in the table. A significantly greater fraction of MLCs were capable of generating ROS compared with both input blasts and residual blasts ($P < 0.01$ for each comparison using the Student's t test). (*C*) pHrodo-green labeled *E. coli* were incubated with input blasts (day 0) and cultured B-ALL cells (day 12) under the conditions listed. These cells were subsequently analyzed for CD19 and CD14 expression as well as pHrodo-green fluorescence by flow cytometry. Phagocytosis of labeled *E. coli* was observed only in MLCs, not in input blasts (*SI Appendix*, Fig. S9*A*) or residual blasts (*SI Appendix*, Fig. S9*B*, dark green); this activity was partially inhibited by cytochalasin D, an inhibitor of phagocytosis.

of genes typically expressed by immature B lymphocytes (Fig. 3 *A* and *B*). Gene sets such as "Kegg_Lysosome" and "Vacuole" were significantly associated with the MLC gene expression profile, consistent with the foamy macrophage-like appearance of these cells (Fig. 2*B*). The most highly down-regulated gene set in MLCs was a B lymphocyte progenitor set, consistent with the transition of MLCs away from an immature B lymphocyte phenotype typical of B-ALL blasts (Fig. 3*B*). Additionally, MLCs down-regulated gene programs associated with telomere maintenance, consistent with a transition to a postmitotic state. The top 10 up-regulated and down-regulated genes in MLCs compared with input blasts in the Molecular Signatures Database (MSigDB) v4.0 collections 2 (curated gene sets) and 5 (GO gene sets) are shown in *SI Appendix*, Fig. S10 *A* and *B*.



NAME	NES	FDR q-val
BROWN_MYELOID_CELL_DEVELOPMENT_UP	2.42	0
LINDSTEDT_DENDRITIC_CELL_MATURATION_D	2.36	0
KEGG_LYSOSOME	2.28	0
MCLACHLAN_DENTAL_CARIES_UP	2.21	0
JAATINEN_HEMATOPOIETIC_STEM_CELL_DN	2.20	0
ICHIBA_GRAFT_VERSUS_HOST_DISEASE_35D_UP	2.19	0
PAPASPYRIDONOS_UNSTABLE_ATEROSCLEROTIC_PLAQUE_UP	2.16	0
RUTELLA_RESPONSE_TO_HGF_UP	2.12	1.17E-04
VACUOLE	2.12	1.04E-04

NAME	NES	FDR q-val
HADDAD_B_LYMPHOCYTE_PROGENITOR	-4.32	0
REACTOME_PACKAGING_OF_TELOMERE_ENDS	-3.26	0
REACTOME_TELOMERE_MAINTENANCE	-3.21	0
REACTOME_MEIOTIC_RECOMBINATION	-3.14	0
REACTOME_CHROMOSOME_MAINTENANCE	-3.08	0
REACTOME_RNA_POL_I_PROMOTER_OPENING	-3.06	0
REACTOME_MEIOSIS	-3.01	0
REACTOME_MEIOTIC_SYNAPSIS	-2.99	0
PYEON_HPV_POSITIVE_TUMORS_UP	-2.99	0
REACTOME_DEPOSITION_OF_NEW_CENPA_CONTAINING_NUCLEOSOMES_AT_THE_CENTROMERE	-2.96	0

Fig. 3. Reprogramming of B-ALL blasts to MLCs is associated with expression of myeloid gene programs and can be recapitulated by forced expression of the myeloid transcription factor C/EBPα or PU.1. (*A* and *B*) RNA was isolated from FACS-purified input blasts and MLCs from five separate BCR–ABL1$^+$ B-ALL cases and subjected to gene expression microarray analysis. Gene set enrichment analysis (GSEA) was used to identify gene sets enriched in B-ALL derived macrophage-like cells (*A*) and input blasts (*B*). The top 10 most highly enriched gene sets are listed, along with normalized enrichment score (NES) and the false discovery rate (FDR) *q* value. (*C*) Mammalian expression vectors encoding the transcription factors Ikaros, RUNX1, PU.1, or C/EBPα were introduced into primary B-ALL cells using nucleofection, and these cells were subsequently cultured in the absence of myeloid cytokines for 4 d followed by analysis of CD14 and CD19 expression. The flow cytometry plots shown are gated on successfully transduced (GFP$^+$) cells. (*D*) Fold change in the percentage of cells expressing CD14 between transduced and nontransduced cells for all six samples tested is shown. Ectopic expression of C/EBPα and PU.1 resulted in significantly more cells expressing CD14 (**$P < 0.01$ and *$P = 0.02$, respectively, using the Mann–Whitney *U* test).

We additionally compared the gene expression profile of residual blasts with MLCs (*SI Appendix*, Fig. S10 *C* and *D*). The gene sets that were enriched in MLCs relative to residual blasts overlapped in many cases with those enriched in MLCs relative to input blasts (e.g., "Lysosome," Vacuole, and "Brown_Myeloid Cell_Development"). This implies that the gene expression profile of residual blasts is similar to the profile seen in input blasts in comparison with the MLCs.

Ectopic Expression of the Myeloid Transcription Factor C/EBPα or PU.1 in B-ALL Blasts Induces Myeloid Reprogramming in the Absence of Myeloid Cytokines. C/EBPα is known to be a key mediator of normal myeloid lineage differentiation and myeloid reprogramming in nonmalignant cell types (8, 19). Consistent with this notion, our microarray data indicated that C/EBPα was 9.7-fold more highly expressed in MLCs than in input blasts (FDR <0.01). Additionally, the myeloid/lymphoid transcription factor PU.1 (encoded by SPI1) has been shown to lead to myeloid reprogramming when expressed in immature B cells, although the effect of this factor was weaker than that seen with C/EBPα (8). Given these findings, we sought to test whether transient expression of C/EBPα or PU.1 in BCR–ABL1$^+$ B-ALL blasts would result in myeloid reprogramming. We used nucleofection to ectopically express C/EBPα, PU.1, or the lymphoid transcription factor Ikaros (encoded by *IKZF1*), in six B-ALL patient samples that were known to undergo myeloid reprogramming in the presence of myeloid cytokines. After nucleofection, these cells were cultured in the absence of myeloid-promoting cytokines for 4 d and then analyzed by flow cytometry for expression of CD14 in the GFP$^+$ transfected cells (Fig. 3*C*). Ectopic expression of PU.1 and C/EBPα, but not Ikaros, significantly increased reprogramming in B-ALL blasts relative to GFP alone (Fig. 3*D*).

Myeloid Reprogramming of B-ALL Blasts to MLCs Eliminates Their Leukemogenicity. Our analysis suggested that MLCs were terminally differentiated and in a postmitotic state; therefore, we predicted that myeloid reprogramming would reduce the ability of B-ALL–derived MLCs to cause disease. To evaluate this hypothesis, we FACS purified input blasts, residual blasts, and MLCs from two B-ALL samples that displayed a reprogramming phenotype and transplanted 5×10^4 cells per mouse into sublethally irradiated nonobese diabetic (NOD)/SCID/IL2R-gamma null (NSG) mice. These mice were then monitored for leukemic engraftment and were humanely killed once morbid as required per protocol. We found that input blasts and residual blasts from samples SU079 and SU312 engrafted mice and eventually led to death (Fig. 4*A*). At time of killing, we found that the bone marrow (Fig. 4 *B* and *C*) of mice that had received input blasts was almost completely composed of CD19$^+$/CD34$^+$/human CD45$^+$ leukemic blasts. In contrast, 9 of the 10 mice transplanted with MLCs were alive and healthy up to 1 y after cell transfer with no human engraftment detected in the bone marrow, which contained normal mouse hematopoietic cells (Fig. 4 *A*–*C*). The one exception exhibited 5% CD19$^+$/CD34$^+$/human CD45$^+$ leukemic blasts in the bone marrow, likely due to low-level contamination of the MLC population with residual blasts after FACS purification. This lack of engraftment by MLCs was not simply an effect of ex vivo culture, as residual blasts readily engrafted and caused disease (Fig. 4*A*). Thus, myeloid reprogramming completely abolishes the leukemogenicity of BCR–ABL1$^+$ B-ALL blasts.

Primary CD14$^+$ Cells from Patients with BCR–ABL1$^+$ B-ALL Frequently Possess the BCR–ABL1 Translocation and Recombined VDJ Loci Identical to the Patient's B-ALL Blasts. Our findings prompted us to question whether blast to macrophage reprogramming occurs to some degree in patients with BCR–ABL1$^+$ B-ALL. To investigate this possibility, we FACS purified primary, unmanipulated monocytes/macrophages from five BCR–ABL1$^+$ B-ALL patient samples in our cohort based on expression of CD14 and CD33 and lack of expression of CD19 and CD34. Contamination of sorted cells by CD19^{high}/CD34^{high} B-ALL blasts was 0.5% or less in all instances (*SI Appendix*, Fig. S11*A*). We then subjected these purified cells to FISH for the BCR–ABL1 translocation and detected positive cells in all five cases with a frequency ranging from 2% to 85% (Fig. 5*A*). Of note, BCR–ABL1$^+$ monocytes/macrophages were detected in samples SU020 and SU086, despite the fact that these samples did not undergo reprogramming in response to myeloid cytokines in vitro (*SI Appendix*, Fig. S1 *A* and *C*).

The aberrant expression of myeloid markers on BCR–ABL1$^+$ B-ALL blasts, termed lineage infidelity, has been described (20). However, whereas surface markers may be susceptible to lineage infidelity, such cells retain the morphology and properties of B-ALL leukemic blasts. We FACS purified CD14$^+$CD11b$^+$ cells from B-ALL patient samples and found they exhibited near-universal myeloid morphologic features, including indented nuclei and abundant cytoplasm (*SI Appendix*, Fig. S12*A*). Moreover,

these $CD14^+CD11b^+$ cells, unlike the $CD19^+$ blasts, were positive for ROS generation by NBT assay (*SI Appendix*, Fig. S12). Additionally, these $CD14^+CD11b^+$ cells did not appreciably express B-cell markers typically implicated in lineage infidelity (*SI Appendix*, Fig. S13).

To further investigate the origin of $CD14^+$ BCR–$ABL1^+$ cells detected in vivo, we FACS purified primary $CD14^+$ cells from three BCR–$ABL1^+$ B-ALL patient samples and sequenced the VDJ region of the Ig heavy chain (Fig. 5*A*). This analysis yielded an estimated VDJ recombination frequency of 56–70% for the three samples analyzed (Fig. 5*B*). This yield was similar to the frequency of BCR–ABL1 positivity by FISH in these patient samples (Fig. 5*A*). In parallel, we sequenced the VDJ region of the blast cells from these same patients and determined that two of the patients' sequence reads were consistent with monoclonal blast expansion, whereas a third (SU536) contained oligoclonal sequences (Fig. 5*C*). Comparison of these sequences indicated that the $CD14^+$ primary cells harbored the same VDJ rearrangement(s) as the corresponding blast cells. The prevalence of specific clones among $CD14^+$ cells was similar to that of the blasts, with the exception of three clones in SU536, which were more common (2–7%) in $CD14^+$ cells, but were rare (<1%) in blasts. Collectively, these findings are consistent with myeloid reprogramming occurring in BCR–$ABL1^+$ B-ALL patients in vivo.

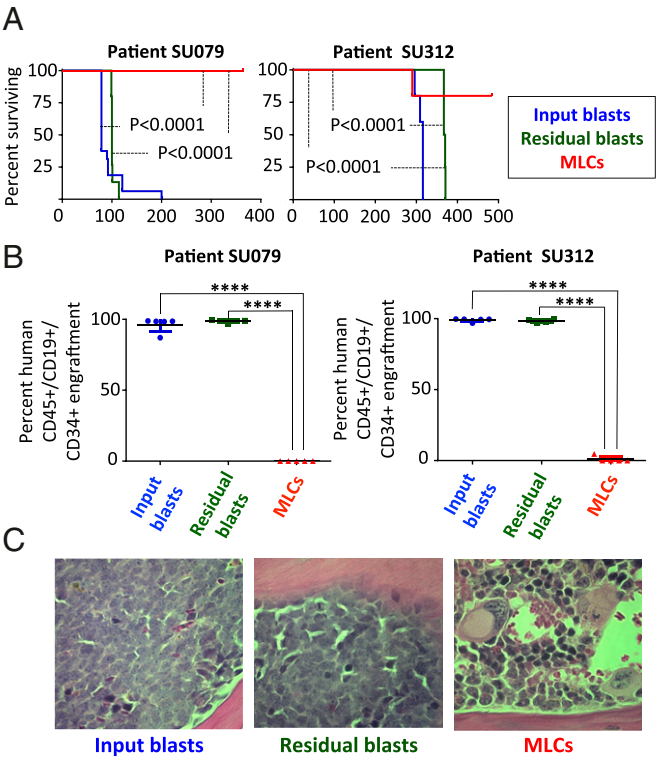


Fig. 4. Myeloid reprogramming of B-ALL blasts to macrophage-like cells eliminates their leukemogenicity. Input blasts (blue), residual blasts (dark green), and MLCs (red) from two B-ALL patients were isolated using FACS and transplanted into NSG mice (5 mice per group for SU312; 15 mice per group for SU079). (*A*) Input blasts and residual blasts caused lethal disease in 100% of mice, whereas only one mouse that received MLCs died (patient SU312). Kaplan–Meier survival analysis is reported (indicated *P* values were calculated using the log-rank test). (*B*) At the first sign of morbidity, mice were killed, and their bone marrow was analyzed for the presence of human leukemia cells. The percentage of total bone marrow cells that were human $CD45^+/CD19^+/CD34^+$ at the time of killing or at the time of last bone marrow aspiration in the case of surviving mice is indicated. ****$P < 0.0001$ by Student's *t* test. (*C*) Representative bone marrow sections of NSG mice that received input blasts, residual blasts, or MLCs (hematoxylin and eosin stain, 1,000×).

A

Patient ID	Fraction of Primary CD14+ Cells Positive for BCR-ABL1 by FISH
SU020	2/100 (2%)
SU079	59/147 (40%)
SU086	10/100 (10%)
SU453	156/200 (78%)
SU536	170/200 (85%)

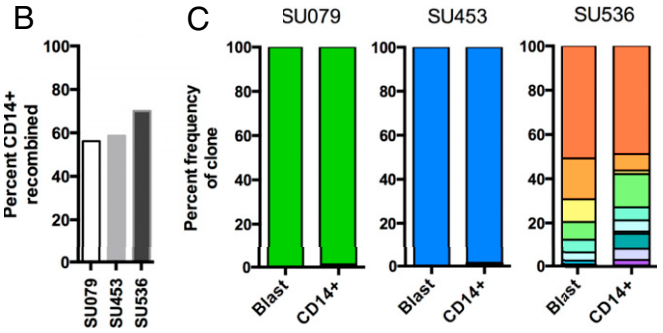


Fig. 5. Primary $CD14^+$ cells from BCR–$ABL1^+$ B-ALL patients frequently possess the BCR–ABL1 translocation and recombined VDJ loci identical to the patient's B-ALL blasts. (*A*) Primary unmanipulated $CD14^+$ monocytes/macrophages were FACS purified from five BCR–$ABL1^+$ B-ALL patient samples based on expression of CD14 and either CD33 or CD11b and lack of expression of CD19 and CD34. These purified monocytes/macrophages were then subjected to FISH for the BCR–ABL1 translocation. (*B*) Similarly purified $CD14^+$ cells were collected from three patients and sequenced at the IgH VDJ locus via a multiplexed PCR assay. The percentage of VDJ-recombined macrophages was calculated by normalizing to the fraction of VDJ sequences retrieved in the cognate blast sample, adjusted for genomic input. (*C*) The relative frequency of each clone is represented by a colored band. The VDJ sequences in primary macrophages from patients SU079 and SU453 possess the same monoclonal sequences as the $CD19^+$ blasts. Additionally, the oligoclonal composition of the SU536 blasts is largely preserved in the macrophages. However, three clones are present in the macrophages at 2–7% frequency (teal, lavender, and purple) that are only present at low (<1%) frequency in the blasts.

Discussion

Recent analysis of B-ALL genomes indicates that mutations in key drivers of B-lymphocyte differentiation contribute to the pathogenesis of this disease, suggesting that strategies to overcome blocked differentiation may yield previously unidentified therapeutic options. We demonstrate here that in some cases, this differentiation block can be overcome via reprogramming to the myeloid lineage. In particular, we show that BCR–$ABL1^+$ B-ALL blasts can be induced to undergo myeloid reprogramming into MLCs upon exposure to myeloid differentiation-promoting cytokines or after ectopic expression of the transcription factor C/EBPα or PU.1. The resultant MLCs are similar to normal human macrophages in many aspects: surface immunophenotype, generation of oxidative burst, and phagocytic capacity. Consistent with the concept that bypassing differentiation blocks can reduce or eliminate the leukemogenicity of these cells, transplanted MLCs do not cause disease in xenograft hosts. Together, our findings establish a rationale for exploiting myeloid reprogramming as a therapeutic strategy in BCR–$ABL1^+$ B-ALL.

Work by Graf and coworkers has demonstrated that immature B cells can be reprogrammed to apparently normal macrophages although enforced expression of C/EBPα (8). Consistent with our findings, this group has also demonstrated that a human B-ALL cell line can be induced to reprogram into macrophages (10). However, robust myeloid reprogramming was seen in only one of

five B-ALL cell lines tested, raising the question as to whether this phenomenon was broadly applicable to primary human B-ALL. We report here the first example to our knowledge of myeloid reprogramming of primary human BCR–ABL1$^+$ B-ALL cells occurring in samples from multiple different patients. Moreover, we demonstrate that myeloid reprogramming can be accomplished through the action of soluble cytokines without genetic manipulation of leukemic cells. These findings suggest that it may be possible to translate myeloid reprogramming into a clinically actionable process applicable to many BCR–ABL1$^+$ B-ALL patients.

As shown in *SI Appendix*, Table S1, BCR–ABL1$^+$ B-ALL samples vary in their capacity to undergo myeloid reprogramming, as we did not detect any evidence of cytokine-induced myeloid reprogramming in 5 of 12 samples tested. The differences in reprogramming capacity could not be explained by the presence or absence of common genomic deletions such as loss of *PAX5* or *IKZF1* (*SI Appendix*, Table S1) or by immunophenotype of the initial patient sample (*SI Appendix*, Table S3). However, gene expression analysis identified an enrichment of genes associated with proliferation (e.g., cell cycle genes) in samples capable of reprogramming in vitro (*SI Appendix*, Fig. S5). We speculate that cells may need to pass through at least one cell division to be reprogrammed, possibly because passage through the cell cycle is required to establish the epigenetic modifications necessary for reprogramming (21). We speculate that such a requirement would not likely pose an insurmountable barrier to the induction of myeloid reprogramming in vivo, as B-ALL cells are highly proliferative (22).

Even after a second round of sorting and culturing B-ALL blasts in reprogramming conditions, a population of residual blasts remains (Fig. 1*B*). This raises the possibility that there is a population of B-ALL blasts in each case that is refractory to reprogramming. However, we speculate that our culture methods are not yet optimized for maximal reprogramming. In particular, our methods are constrained by the limited ability of primary human B-ALL cells to survive long-term ex vivo. Additionally, multiple rounds of sorting and reculturing stress primary cells.

We have shown that myeloid reprogramming of B-ALL cells can be induced ex vivo, raising the question as to whether myeloid reprogramming can occur in vivo in patients. Several studies have demonstrated the presence of BCR–ABL1$^+$ myeloid cells in B-ALL patients (23, 24). These observations have been attributed to the presence of the BCR–ABL1 translocation in a multipotent progenitor cell, capable of both lymphoid and myeloid differentiation. Alternatively, we show here that these BCR–ABL1$^+$ myeloid cells may instead form via reprogramming of B-ALL blasts. The recovery of VDJ reads from primary CD14$^+$ cells that closely reflect the clonal architecture of CD19$^+$ blasts is consistent with lineage reprogramming of blasts into MLCs occurring in vivo. Thus, myeloid reprogramming may occur to some degree in patients, although it is unknown if this phenomenon can impact the clinical course of high-risk B-ALL. However, if myeloid reprogramming can be accelerated in vivo, it might be possible to exploit this phenomenon to therapeutic benefit.

Materials and Methods

Materials and methods are presented in *SI Appendix, SI Materials and Methods*. Included are detailed descriptions of: the collection of human samples; flow cytometry and cell sorting; cell culture conditions; fluorescence in situ hybridization; IgH chain gene clonality assay; NBT assay; phagocytosis assay; RNA purification, amplification, and microarray analyses; assessment of genomic DNA copy number abnormalities; ectopic expression of transcription factors; and animal care and NSG xenotransplantation assay. Human samples were obtained from patients at the Stanford University Medical Center with informed consent, according to Institutional Review Board (IRB) approved protocols (Stanford IRB nos. 6453 and 18329). All mouse experiments were conducted according to an Institutional Animal Care and Use Committee approved protocol and in adherence to the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (25).

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