Targeting the MYC and PI3K Pathways Eliminates Leukemia-Initiating Cells in T-cell Acute Lymphoblastic Leukemia

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Abstract

Disease relapse remains the major clinical challenge in treating T-cell acute lymphoblastic leukemia (T-ALL), particularly those with PTEN loss. We hypothesized that leukemia-initiating cells (LIC) are responsible for T-ALL development and treatment relapse. In this study, we used a genetically engineered mouse model of Pten−/− T-ALL with defined blast and LIC-enriched cell populations to demonstrate that LICs are responsible for therapeutic resistance. Unlike acute and chronic myelogenous leukemia, LICs in T-ALL were actively cycling, were distinct biologically, and responded differently to targeted therapies in comparison with their differentiated blast cell progeny. Notably, we found that T-ALL LICs could be eliminated by cotargeting the deregulated pathways driven by PI3K and Myc, which are altered commonly in human T-ALL and are associated with LIC formation. Our findings define critical events that may be targeted to eliminate LICs in T-ALL as a new strategy to treat the most aggressive relapsed forms of this disease. Cancer Res; 74(23); 7048–59. ©2014 AACR.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a common hematologic malignancy that is associated with a significant risk of disease relapse and poor prognosis (1). Activating mutations in NOTCH1 are present in approximately half of the patients with T-cell leukemia (2), and deletion or mutations of the PTEN tumor suppressor gene have been reported in 8% to 63% of pediatric T-ALL patients (3–6) and are correlated with poor prognosis (7, 8). Furthermore, PTEN is frequently inactivated nongenetically in primary T-ALL cells (9) and constitutive activation of the NOTCH signaling pathway downregulates PTEN expression (6, 10), suggesting that PTEN and its controlled PI3K/AKT/mTOR pathway are critical for the etiology of human T-ALL.

Individual cells within tumor masses exhibit notable heterogeneity in their functional and pathologic properties. Recent evidence supports the model that the growth of many types of cancers, including leukemia, is sustained by a subset of cells, termed cancer stem cells (CSC), that are responsible for the initiation and propagation of the disease and show ability to initiate cancer in xenografts or genetically engineered mouse models (11–14). CSCs are characterized by their self-renewal capacity and ability to generate all cell types that comprise the cancer, and are functionally distinct from the bulk of tumor cells that lack the ability to initiate tumor growth. A consequence of this model is that therapies that only eliminate the bulk of the tumor cells without targeting CSCs will result in re-emergence of disease (15).

In hematopoietic malignancies, frequent relapse following conventional chemotherapies suggests that leukemia-initiating cells (LIC) are spared by the treatment, which may be attributed to properties shared with normal hematopoietic stem cells such as maintenance of a quiescent state (16–18). In acute myelogenous leukemia (AML), most LICs isolated from patient samples are quiescent (18) and protected from chemotherapeutic agents (19, 20). Maintenance of a quiescent state has also been associated with LICs in chronic myelogenous leukemia (CML) and resistance to tyrosine kinase inhibitors (21, 22). However, the identity and cell-cycle status of LICs in T-ALL has not been explored to date. To understand the cellular and molecular basis of T-ALL relapse and develop more effective therapies, there is a need to characterize T-ALL LICs using functional assays and determine the relationship between LICs and disease relapse in models that mimic human T-ALL pathogenesis.

We previously generated a VE-Cadherin-Cre+;PtenloxP/loxP, Rosa26Rosa26LOxSTOP-LacZ (Pten-null) T-ALL model to investigate the molecular mechanisms underlying Pten inactivation-mediated leukemogenesis. In this model, disease is initiated by the conditional deletion of Pten in the fetal liver hematopoietic stem cells and animals develop T-ALL with 100% penetrance in the absence of activating Notch1 mutations (23). In addition to...
additional cell line authentication was performed. Jurkat, York, NY; MOLT-3, MOLT-4, and T-cell lymphoma line at UCLA (Jurkat), A. Ferrando at Columbia University (New Human T-ALL lines were generously provided by Drs. G. Cheng et al). Sodium pyruvate, glutamine, penicillin, and streptomycin (all from Peprotech), 10 mmol/L HEPES, nonessential amino acids, and antibiotics were added to the culture medium. Mice and transplantation assays BALB/c mixed background mice were generated as previously described (23). NOD-SCID-IL2Rγc−/− (NSG) mice were obtained from UCLA’s Defined Flora Mouse Facility. 10^7 Pten-null T-ALL BM or splenocytes harvested from primary Pten-null T-ALL mice were transplanted by tail vein injection into nonirradiated NSG recipients as previously described (23) to generate cohorts of mice with T-ALL to perform analyses. To evaluate LIC activity after drug treatment, bone marrow (BM) was harvested from 7- or 14-day treated mice and transplanted into secondary NSG recipients at 10^7 to 10^8 cell doses. All animal experiments were approved by the University of California, Los Angeles (UCLA: Los Angeles, CA) Animal Research Committee and conducted according to relevant regulatory standards.

Rapamycin, JQ1, and VX-680 in vivo treatment Mice were administrated the following single agents i.p. daily for 7 or 14 days: 4 mg/kg rapamycin (LC Laboratories), 50 mg/kg JQ1 (Bradner Laboratory, Dana-Farber Cancer Institute; Boston, MA), or 75 mg/kg VX-680 (I.C Laboratories). For combination treatments, mice were treated daily for 7 or 14 days with 4 mg/kg rapamycin and 50 mg/kg JQ1 or 4 mg/kg rapamycin and 40 mg/kg VX-680. At indicated time points, BM and spleen were harvested for pathologic and flow-cytometric analysis.

Cell lines Pten-null T-ALL cells were derived from primary thymocytes harvested from a Pten-null mouse with T-ALL and maintained in DMEM (Life Technologies) supplemented with 20% FBS (Omega Scientific), 10 ng/ml IL-7 (both Peprotech), 10 mmol/L HEPES, nonessential amino acids, sodium pyruvate, glutamine, penicillin, and streptomycin (all Life Technologies), and 2-mercaptoethanol (BME; Sigma). Human T-ALL lines were generously provided by Drs. G. Cheng at UCLA (Jurkat), A. Ferrando at Columbia University (New York, NY; MOLT-3, MOLT-4, and T-cell lymphoma line CUTT11), C. Radu at UCLA (CCRF-CEM), and X. Chen at the University of Maryland (College Park, MD; MOLT-16). No additional cell line authentication was performed. Jurkat, CCRF-CEM, MOLT-3, and MOLT-4 cells were maintained in RPMI (Life Technologies) supplemented with 10% FBS, glutamine, penicillin, and streptomycin. MOLT-16 and CUTT11 cells were maintained as the other human cell lines but supplemented with 20% FBS.

Proliferation and viability assays Cell viability was assessed by measuring the conversion of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Life Technologies) to formazan or by using the CellTiter 96 AQueous One Solution Cell Proliferation Assay (Promega). Cells were seeded 24 hours before drug addition. After 48-hour incubation with drug or appropriate vehicle (DMSO or ethanol), MTT or CellTiter 96 AQueous One Solution Assay reagents were added as specified in reagent protocols and absorbance was read using a Bio-Rad Benchmark microplate spectrophotometer.

Statistical analysis The difference between experimental groups was compared using Student t test analysis. Survival in animal experiments was represented with Kaplan–Meier plots (GraphPad Prism). IC50 was calculated using Graphpad Prism.

Supplementary data Supplementary Data include Supplementary Materials and Methods, seven Supplementary figures, and one Supplementary Table.

Results Leukemia-initiating cells are responsible for therapeutic resistance in Pten-null T-ALL We previously found that rapamycin, a specific TORC1 inhibitor, abrogated LIC formation by preventing the Tcra/δ-c-Myc translocation, a critical secondary event for Pten-null T-ALL development in this model (24). However, when treatment began at the acute T-ALL stage, rapamycin extended survival but all Pten-null animals eventually succumbed to T-ALL (24). To understand the cellular and molecular mechanisms underlying relapse, we treated Pten-null T-ALL mice with rapamycin for 7 days, harvested BM cells, and transplanted these cells into secondary and tertiary NOD-SCID-IL2Rγc−/− (NSG) recipients in the absence of drug. We then retreated the transplant recipients with rapamycin to test whether rapamycin-treated T-ALL remained sensitive to the drug or acquired resistance mutations (Fig. 1A). Rapamycin significantly reduced the percentage of phospho-S6^R cell populations, a surrogate marker for mTOR activity, in secondary and tertiary recipients from greater than 70% to 15% after 2 days of retreatment, suggesting that the majority of T-ALL cells remained sensitive to rapamycin (Fig. 1B). In addition, we did not detect “hotspot” mutations in Notch1 or Fbxw7, common alterations found in human T-ALL, after treatment (Fig. 1C). Importantly only LICs, not blasts, harvested from tertiary retreated animals could efficiently initiate T-ALL upon transplantation (Fig. 1D). Altogether these data suggest that resistance to rapamycin is due to failure to eliminate LICs and not mutations in the mTOR or NOTCH pathways.
Cotargeting deregulated PI3K and Myc efficiently eliminates Pten-null LICs

The deregulated c-Myc oncogene, either via Tcrα/β-c-Myc translocation or through its overexpression, is essential for LIC formation and leukemia development in the Pten-null T-ALL model (24) and associated with LICs in NOTCH1-induced T-ALL (25). Therefore, we hypothesized that combination therapy that cotargets the PI3K pathway and deregulated c-Myc might eliminate LIC and T-ALL. The bromodomain and extraterminal subfamily of bromodomain proteins (BRD2, BRD3, and BRD4) has emerged as potent regulators of MYC expression in various tumor types, and the bromodomain inhibitor JQ1 has been demonstrated to downregulate MYC transcription, reduce expression of MYC target genes, and show antileukemic effects in vitro and in vivo models (26–30).

To test whether JQ1 can be used in combination with rapamycin for therapeutic utility, we transplanted leukemic cells from primary Pten-null T-ALL into NSG recipients to generate a cohort of Pten-null T-ALL animals to perform parallel analyses of treatment with agents, alone or in combination (Supplementary Fig. S1A). To determine the specific responses of blasts and LICs, we quantified the percentages of phospho-S6+= cells in Pten-null T-ALL blast cells in BM harvested from mice after 2 days of rapamycin treatment in vivo (4 mg/kg daily) using intracellular flow-cytometric analysis. C, summary of mutational analysis of Notch1 exons 26, 27, and 34 and Fbxw7 exons 8, 9, and 10 using genomic DNA from splenocytes harvested from T-ALL mice after single or double rapamycin treatment. Ratios indicate number of T-ALL mice with mutations (Mut) over total number T-ALL mice screened. D, summary of transplantation assay with sorted fractions. Blast and LIC sorted populations were transplanted into NSG recipients at indicated doses and leukemia development was evaluated in recipients. Data in B are represented as mean ± SEM.
in this compartment (Supplementary Fig. S1B). JQ1 significantly enhanced the effects of rapamycin in reducing splenomegaly and almost completely eliminated leukemic blasts after 7 days of daily in vivo treatment (Fig. 2A; left two). Importantly, the percentage of LICs in the BM was markedly reduced with the combination treatment (Fig. 2A; right). JQ1 single-agent treatment, however, did not effectively eliminate blast cells in vivo, as JQ1 single treated animals appeared weak during treatment and showed splenomegaly upon sacrifice (data not shown). To assess the functional significance of the reduction in LICs with combination treatment, we performed limiting dilution transplantation analyses using 10^3 to 10^5 BM cells harvested from 7-day treated animals (Supplementary Fig. S1A). Although all animals receiving a 10^3 cell dose of rapamycin-treated cells succumbed to T-ALL by approximately 30 days, none of the animals receiving the same dose of dual treated cells developed any sign of illness over more than 100 days (Fig. 2B) and no leukemia cells could be detected in the euthanized animals using sensitive genomic PCR analysis (Supplementary Fig. S1C). Our regression analysis showed that combination treatment reduced the frequency of the LIC to approximately 1/200,000 (Fig. 2C), indicating that eliminating LIC number and activity is directly associated with this remarkable therapeutic response.

T-ALL may be stratified and treated according to its molecular and genetic defects

The robust response of our Pten-null T-ALL model to combination treatment of rapamycin and JQ1 prompted us to investigate the molecular mechanisms underlying T-ALL elimination and to test whether similar combination treatment can be applied to human T-ALL. For this, we generated a Pten-null T-ALL in vitro culture system to circumvent the limitation of the in vivo model in conducting cellular, biochemical, and pathway analyses. By optimizing culture conditions that allow for the survival and expansion of primary leukemia cells harvested from Pten-null T-ALL mice, we established Pten-null T-ALL cell lines that require critical T-cell cytokines IL2 and...
IL7 for growth, are CD3:\textsuperscript{+},TCR\textsuperscript{+},LacZ\textsuperscript{+}, express high levels of c-Myc, and retain leukemia-initiating activity in vivo upon transplantation (Schubbert and colleagues; manuscript in preparation).

To assess the therapeutic utility of rapamycin and JQ1 in human T-ALLs, we first investigated the effect of these drugs on the growth and viability of our newly developed Pten-null T-ALL line in comparison with several human Pten-null T-ALL lines. Interestingly, we found that MOLT-16 cells, which harbor similar genetic alterations (Pten null with a Tcra-c-Myc translocation, lacking p53 mutations and the activated, intracellular form of NOTCH1; ref. 31) as our Pten-null T-ALL model, are very sensitive to both rapamycin and JQ1 (Fig. 3A and Supplementary Figs. S2A and S3A). Other human Pten-null T-ALL lines, including those with NOTCH1 and p53 mutations, are less sensitive to rapamycin and JQ1, with Jurkat cells being most resistant (Fig. 3A and Supplementary Figs. S2A and S3A). Further analysis of Pten-null T-ALL cells revealed that rapamycin caused accumulation of cells in G\textsubscript{1} and a rapid and robust decrease in cell size and downregulation of the surface expression of the transferrin receptor CD71 and amino acid transporter CD98 (Fig. 3B and C and Supplementary Fig. S2B). mTOR activity regulates nutrient transporter levels in Pten-null T-ALL (24) and the control between mTOR activity and nutrient sensing and uptake may play a critical role for cell survival, proliferation, and differentiation (32). Jurkat cells were less sensitive to reduction in cell size by rapamycin and levels of nutrient receptor expression remained high after treatment (Fig. 3C). Similarly, JQ1 significantly downregulated c-Myc protein levels and induced apoptosis-associated cleavage of PARP and caspase-3 in Pten-null T-ALL while only moderately reduced c-Myc levels were observed in Jurkat cells in the absence of apoptosis (Fig. 3D and Supplementary Fig. S3B). Interestingly, MOLT-16 cells were markedly more sensitive to JQ1-mediated induction of apoptosis compared with other human T-ALL lines tested (Supplementary Fig. S3B and S3C). The induction of apoptosis in Pten-null T-ALL and MOLT-16 in response to JQ1 occurred following marked G\textsubscript{1} arrest (Supplementary Fig. S3D) and was not associated with upregulation of p53 (Supplementary Fig. S3C).

We investigated the efficacy of rapamycin and JQ1 treatment in four human pediatric T-ALL samples using in vitro growth and viability assays. Interestingly all four human T-ALL samples showed sensitivity to rapamycin but were less sensitive to JQ1 (Supplementary Fig. S4A and S4B). No \textit{Pten} mutations were found in any of the human samples (Supplementary Table S1), but phospho-AKT levels were similar or greater than Pten-null Jurkat cells and at least 2-fold greater than CUTLL1 cells, a PTEN-positive human T-cell lymphoma line (Supplementary Fig. S4C). The expression of p53 and Bcl-2 was increased in Jurkat cells in response to 100 nmol/L rapamycin at 24 hours. D, immunoblot analysis for c-Myc, PARP, and cleaved caspase-3 (c. caspase-3) in Pten-null T-ALL and Jurkat cells after 24-hour JQ1 treatment at indicated doses. Data in A through C are represented as mean \pm SEM.
downstream targets (9, 34). Levels of c-Myc in the primary human T-ALL samples were low in comparison with Jurkat cells (Supplementary Fig. S5A and S5B), which may in part explain the weak response to JQ1.

**LICs and leukemic blasts are biologically distinct and respond differently to targeted therapies**

The remarkable responses of blast and LIC populations to rapamycin and JQ1 combination treatment in Pten-null T-ALL in vivo prompted us to examine the specific effects of these agents in these distinct compartments. We hypothesized that the failure of rapamycin as a single agent to eliminate Pten-null LICs in vivo may be due to the differential cellular and biochemical responses of LICs and blasts to rapamycin. Indeed, we found that a 2-day treatment with rapamycin in vivo greatly reduced levels of phospho-S6 in the blast cell compartment, whereas levels of phospho-S6 remained high in LICs (Fig. 4A). Furthermore, we found that Pten-null LICs are markedly less sensitive to the effects of rapamycin on reducing cell size and nutrient receptor levels in comparison with blast cells (Fig. 4B). These data suggest that failure to inhibit mTOR and effectively downregulate nutrient receptors in the LIC compartment may underlie the mechanism of rapamycin resistance.

Because Myc is associated with LIC activity in both our model and NOTCH1-driven T-ALL, we evaluated the effect of JQ1 on Myc levels in the blasts and LIC-enriched compartments. Although JQ1 reduced levels of c-Myc in both blast and LIC populations, we found that LICs are significantly more sensitive to JQ1-mediated downregulation of c-Myc than their blast progenies (Fig. 4C; 18% vs. 49% c-Myc+ in LICs and blasts, respectively, after 500 nmol/L JQ1 treatment). Quantitative analysis revealed that LICs show 2- to 3-fold greater reduction in c-Myc levels after JQ1 treatment compared with blast cells (Fig. 4D). The enhanced sensitivity of Pten-null LICs to c-Myc downregulation by JQ1 may be particularly valuable to efficiently target aberrant c-Myc levels in Pten-null LICs and eliminate these cells. Taken together, these data demonstrate that LIC and blast subpopulations are not only functionally distinct but respond differently to targeted treatments.

**LICs in Pten-null T-ALL are actively cycling**

The proto-oncogene c-Myc is a key regulator of cell growth and cell-cycle progression (35). Because Pten-null LICs over-express c-Myc, we evaluated the cell-cycle status of LICs. Intriguingly, when we measured bromodeoxyuridine (BrdUrd) incorporation in the BM of primary and transplanted Pten-null LICs in Pten-null T-ALL are actively cycling.
T-ALL \textit{in vivo}, we found that a large portion of LICs was actively cycling (~50% BrdUrd \textsuperscript{+} after 1-hour pulse labeling) similar to blast cells (Fig. 5A and quantified in 5B). This observation contrasts with studies of LICs in other hematopoietic malignancies, including AML and CML, in which the LICs reside mostly in a quiescent state (13, 19, 21), however, is similar to the increased levels of proliferation and self-renewal reported for \textit{Nras} \textsuperscript{G12D} mutant hematopoietic stem cells (36, 37).
Our cell-cycle analysis suggests that deregulated \textit{Myc} expression in T-ALL LICs may promote their cell-cycle entry and progression, which distinguishes T-ALL LICs from AML and CML LICs. Consistent with this notion, we found that 86% of cells with high c-Myc levels (Myc\textsuperscript{high}) are also BrdUrd\textsuperscript{+}, whereas only 33% of cells with low c-Myc levels (Myc\textsuperscript{low}) are BrdUrd\textsuperscript{+} (Fig. 5C). Importantly, JQ1 treatment could effectively eliminate the Myc\textsuperscript{high} population (Fig. 5D), supporting the idea that high c-Myc levels in the LIC subpopulation may drive LIC entry into the cell cycle. These unique characteristics of Pten-null T-ALL LICs may render them sensitive to cell-cycle inhibitors.

\textbf{Ptn-null T-ALL LICs can be effectively eliminated by Aurora kinase inhibition in combination with rapamycin}

Myc upregulation of Aurora kinases, which have essential roles in many aspects of cell division (38, 39), is reported to be essential for the maintenance of Myc-driven malignancies (40). Interestingly, we found that JQ1-induced c-Myc downregulation is associated with a significant reduction of Aurora B levels (Fig. 6A), suggesting that c-Myc regulates Aurora B in Pten-null T-ALL. This node may be critical for leukemia maintenance and further implicates potential utility of cell-cycle inhibitors to eradicate LICs.

In support of this hypothesis, the Aurora kinase inhibitor VX-680 has been reported to show synthetic lethality with Myc upregulation of Aurora kinases, which have essential roles in many aspects of cell division (38, 39), is reported to be essential for the maintenance of Myc-driven malignancies (40). Interestingly, we found that JQ1-induced c-Myc downregulation is associated with a significant reduction of Aurora B levels (Fig. 6A), suggesting that c-Myc regulates Aurora B in Pten-null T-ALL. This node may be critical for leukemia maintenance and further implicates potential utility of cell-cycle inhibitors to eradicate LICs.

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and p21 in E). We also detected polyploidy in Jurkat cells, it does not induce apoptosis as apoptosis (Fig. 7E). Although VX-680 causes mitotic block and may be involved in the DNA damage response and subsequent presence of a molecularly deregulated Myc. 

human T-ALL model with functionally defined and distinguishable blast and LIC-enriched populations provides a valuable platform to develop novel targeted molecular therapies in which efficacy can be evaluated in both LICs and the bulk of the disease. Our biochemical and molecular analysis of drug effects on the blast and LIC-enriched compartments revealed that LICs and blasts show remarkably distinct responses to targeted therapies. Although the bulk of Pten-null T-ALL shows rapid and robust inhibition of the mTOR pathway by rapamycin, even after multiple rounds of therapy, rapamycin fails to inhibit mTOR and downregulate nutrient receptor expression in the LIC compartment, which retains LIC activity after treatment. These data suggest that rapamycin is caused by the failure to eliminate LICs.

Clinical data suggest that conventional chemotherapies may reduce the "bulk" of the leukemia (blast cells), but fail to eradicate the LICs, leading to relapse (15). In this study, our Pten-null T-ALL model with functionally defined and distinguishable blast and LIC-enriched populations provides a valuable platform to develop novel targeted molecular therapies in which efficacy can be evaluated in both LICs and the bulk of the disease. AML and CML reside in a quiescent state (13, 19, 21), to our knowledge, the cell-cycle status of LICs in T-ALL has thus far never been characterized. We demonstrated that Pten-null T-ALL LICs (i) are active in the cell cycle, which is most likely driven by the Tcrα/β-c-Myc translocation induced c-Myc overexpression; (ii) are responsible for therapeutic resistance; (iii) are biologically distinct and respond differently to targeted therapies in comparison with their differentiated progeny; and (iv) can be eliminated by cotargeting deregulated PI3K and Myc pathways, two common alterations found in human T-ALL that are associated with the formation of LICs (Fig. 7F). Our study further demonstrates that LICs are addicted to the molecular and genetic events associated with their formation and cotargeting these events may provide a potent strategy to reduce leukemia burden and overcome LIC-mediated therapeutic resistance.

VX-680 causes mitotic block, polyploidy, and apoptosis in Pten-null T-ALL

We used our in vitro model to evaluate the cellular and biochemical effects of VX-680 on Pten-null T-ALL and determine potential mechanisms underlying T-ALL elimination. Pten-null T-ALL cells were highly sensitive to VX-680 (IC50 ~83 nmol/L) and treatment decreased the levels of phosphorylation of Histone H3 at Ser10, a surrogate for Aurora B kinase activity, within 8 hours (Fig. 7A). We further tested the sensitivity of several human Pten-null T-ALL lines to VX-680. Consistent with the notion that Aurora B is a major target of c-Myc function, sensitivity to VX-680 mirrored that of JQ1 with MOLT-16 cells, harboring similar genetic alterations as Pten-null T-ALL, showing greatest sensitivity, and Jurkat cells, harboring NOTCH1 (43) and p53 mutations, most resistant to treatment (Fig. 7B). Further analysis demonstrated that VX-680 treatment caused a block in mitosis and induced apoptosis in Pten-null T-ALL as indicated by accumulation of cells in G2-M, formation of a polyploid population (8N), and a pronounced increase in the sub-G1 population with induction of cleaved PARP and caspase-3 (Fig. 7C–E). We also detected increased levels of phosphorylated H2AX and induction of p53 and p21 in Pten-null T-ALL, suggesting that the p53 pathway may be involved in the DNA damage response and subsequent apoptosis (Fig. 7E). Although VX-680 causes mitotic block and polyploidy in Jurkat cells, it does not induce apoptosis as illustrated by the lack of a sub-G1 population and absence of cleaved PARP and caspase-3 (Fig. 7C–E), possibly due to the presence of a p53 mutation. These results suggest that therapeutic approaches using VX-680 may be most effective in human T-ALL with PTEN loss, functional p53, and genetic or molecularly deregulated Myc.

Discussion

Our work provides insight into the molecular and cellular mechanisms underlying relapse in Pten-null T-ALL and uncovers novel biologic and functional properties of LICs that can be exploited for therapeutic intervention. We demonstrate that Pten-null T-ALL LICs (i) are active in the cell cycle, which is most likely driven by the Tcrα/β-c-Myc translocation induced c-Myc overexpression; (ii) are responsible for therapeutic resistance; (iii) are biologically distinct and respond differently to targeted therapies in comparison with their differentiated progeny; and (iv) can be eliminated by cotargeting deregulated PI3K and Myc pathways, two common alterations found in human T-ALL that are associated with the formation of LICs (Fig. 7F). Our study further demonstrates that LICs are addicted to the molecular and genetic events associated with their formation and cotargeting these events may provide a potent strategy to reduce leukemia burden and overcome LIC-mediated therapeutic resistance.

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T-ALL LICs and offer therapeutic benefits to eliminate T-ALL. Indeed, rapamycin in combination with the Aurora kinase inhibitor VX-680 effectively reduced Pten-null LICs and T-ALL in vivo. Although we attribute the active cycling of Pten-null LICs to deregulated Myc expression, it is possible that activation of other signaling pathways may also contribute to cycling. Notably, NrasG12D-mutant hematopoietic stem cells have been recently reported to show increased proliferation and self-renewal (37).

Our study provides support that LICs are responsible for relapse and uncovers a role for anti–MYC-directed therapies to target LICs, similar to a recent study (44). We also reveal a role for rapamycin as an effective agent to rapidly reduce the bulk of the leukemia, the blast population. The most effective anticancer regimens will likely involve combination approaches using therapies that kill both bulk tumor cells and disease-initiating cells (15). Our novel combination treatment strategy using agents to target the PI3K pathway and deregulated Myc is consistent with this paradigm to provide anticancer therapies that quickly reduce disease burden and eliminate the root of the cancer.

Loss of PTEN and deregulation of Myc are common in human T-ALL and associated with the formation of LICs (3–6, 23, 25). The remarkable efficacy of cotargeting the PI3K
pathway and Myc to eliminate Pten-null/Teratagenesis suggests that LICs are addicted to molecular events critical for their formation and can be effectively eliminated by targeting these alterations. The sensitivity of our human pediatric T-ALL samples with activated AKT to rapamycin supports this hypothesis and examination of primary human T-ALL samples with c-Myc translocation, similar to the MOLT-16 patient-derived cell line, will further our understanding of patient response. Overall our study strongly suggests that human T-ALL needs to be stratified by the molecular genetic events associated with LIC formation and treated by cagerting pathways of addiction.

Disclosure of Potential Conflicts of Interest

J.E. Bradner is a consultant/advisory board member for Tensha Therapeutics. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: S. Schubbert, H. Wu
Development of methodology: J.E. Bradner
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A. Cardenas, H. Chen, C. Garcia
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Cardenas, H. Chen, J.E. Bradner, H. Wu

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