Acute T-cell leukemias remain dependent on Notch signaling despite PTEN and INK4A/ARF loss

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NOTCH1 is activated by mutation in more than 50% of human T-cell acute lymphoblastic leukemias (T-ALLs) and inhibition of Notch signaling causes cell-cycle/growth arrest, providing rationale for NOTCH1 as a therapeutic target. The tumor suppressor phosphatase and tensin homolog (PTEN) is also mutated or lost in up to 20% of cases. It was recently observed among human T-ALL cell lines that PTEN loss correlated with resistance to Notch inhibition, raising concern that patients with PTEN-negative disease may fail Notch inhibitor therapy. As these studies were limited to established cell lines, we addressed this issue using a genetically defined mouse retroviral transduction/bone marrow transplantation model and observed primary murine leukemias to remain dependent on NOTCH1 signaling despite Pten loss, with or without additional deletion of p16ink4a/p19arf. We also examined 13 primary human T-ALL samples obtained at diagnosis and found no correlation between PTEN status and resistance to Notch inhibition. Furthermore, we noted in the mouse model that Pten loss accelerated disease onset and produced multiclonal tumors, suggesting NOTCH1 activation and Pten loss may collaborate in leukemia induction. Thus, in contrast to previous findings with established cell lines, these results indicate PTEN loss does not relieve primary T-ALL cells of their “addiction” to Notch signaling. (Blood. 2010;115:1175-1184)

Introduction

The 4 mammalian Notch genes (NOTCH1-4) encode a family of highly conserved type I transmembrane receptors that are normally activated by ligands of the Delta/Serrate/Lag-2 family. Although regulated NOTCH1 signaling is important for normal T-cell development,1 it is frequently activated by mutation in the human cancer T-cell acute lymphoblastic leukemia (T-ALL).2 The potent oncogenicity of activated NOTCH1 has been demonstrated in murine bone marrow transplantation/transplantation models and several transgenic mouse lines.3

Activating NOTCH1 mutations occur in more than 50% of primary human T-ALLs and cluster in the heterodimerization (HD) and C-terminal proline-, glutamic acid-, serine-, and threonine-rich (PEST) domains.4 HD mutations result in weakened association or complete dissociation of the receptor subunits, and thus lead to heightened/constitutive activation of the receptor.5 PEST domain mutations often generate premature stop codons that delete the PEST degron, and thus enhance signaling by reducing turnover/prolonging half-life of activated ICN.4 When present together, the HD and PEST mutations occur in cis, and stimulate signaling in a synergistic fashion.4 Interestingly, a similar overall frequency of Notch1 mutations (mostly PEST, but some HD) has been observed in various mouse models of T-ALL, underscoring the importance of Notch1 signaling in T-cell leukemogenesis. In addition, both human and murine T-ALL cells bearing NOTCH1 mutations are frequently sensitive to treatment with inhibitors of Notch signaling including γ-secretase inhibitors (GSIs) that induce G1 cell-cycle/growth arrest and in some cases apoptosis.4,7-10 Based on these findings, GSIs and other inhibitors of Notch signaling are being pursued for clinical use in patients with T-ALL.11

The phosphatidylinositol 3-kinase (PI3K)/phosphatase and tensin homolog (PTEN) pathway is one of the most commonly mutated signaling pathways in human cancer.12 Whereas PI3K activates downstream signaling through AKT by converting phosphatidylinositol 4,5-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-triphosphate (PIP3), PTEN antagonizes PI3K activity by converting PIP3 back to PIP2. Pten-deficient mice are embryonically lethal, but heterozygotes display high incidence of spontaneous T-cell leukemia/lymphoma associated with loss of heterozygosity of the wild-type allele.13 Similarly, T-cell leukemia/lymphoma occurs in various conditional Pten-null models13,16 as well as in mice that received a transplant of Pten-deficient adult hematopoietic stem cells,17 further supporting an important contributing role for PI3K pathway activation in T-cell transformation. Finally, rapamycin can inhibit the serial transplantability of murine...
acute myeloid leukemia/T-ALL, suggesting a role for PI3K pathway activation in leukemia stem cell maintenance.17,18

PTEN protein loss is observed in 17% to 20% of human T-ALL cases, with chromosomal rearrangements involving the PTEN locus occurring in 15% and frameshift/truncating mutations in 5% to 8% of cases.19,21 More strikingly, more than 85% of cases exhibit hyperactivated PI3K/AKT signaling, which was correlated with reduced PTEN protein phosphatase activity either by casein kinase 2-mediated phosphorylation and/or reactive oxygen species–dependent oxidation.22 Interestingly, it was recently observed among human T-ALL cell lines that loss of PTEN was strongly correlated with GSI resistance, suggesting constitutive PI3K/AKT signaling could relieve dependence on Notch signaling.20 Because Notch inhibitors are currently being tested in patients with T-ALL and other cancers in which PTEN may be lost or mutated, we felt it was important to confirm whether PTEN loss indeed conferred resistance to NOTCH1 inhibition using primary T-ALL cells as opposed to established cell lines.

To this end, we generated primary murine leukemias on Pten-null versus wild-type backgrounds using a standard retroviral transduction/bone marrow transplantation approach. Given that most human T-ALLs lack CDKN2A, which encodes p16INK4A/CDKN2A and other cancers in which PTEN may be lost or mutated, we felt it was important to confirm whether PTEN loss indeed conferred resistance to NOTCH1 inhibition using primary T-ALL cells as opposed to established cell lines.

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In vitro BrdU incorporation assay
Cultured cells were pulsed with 10μM bromodeoxyuridine (BrdU) for 2 hours in vitro prior to harvest. Staining was performed according to the manufacturer’s instructions (allophycocyanin- or fluorescein isothiocyanate–BrdU kit; BD Biosciences). Human leukemia cells from xenografted mice were discriminated using anti–human CD45 antibody (eBioscience). Murine leukemia cells were discriminated by virtue of retroviral GFP expression.

RT-PCR
Total RNA was prepared using TRizol reagent (Invitrogen). Reverse transcription was performed with SuperScript II RT (Invitrogen) on a Dyad Disciple thermal cycler (Bio-Rad). Gene-specific primer sequences were as follows: human Pten forward 5- GTT TAC CGG CAG CAT CAA AT, human Pten reverse 5-CCC CCA CTT TAG TGC ACA GT; human p14ARF forward 5- TCG TGC TGA TGC TAC TGA GG, human p16INK4A forward 5-CAA CGC ACC GAA TAG TTA CG, human p16INK4A/p14ARF reverse 5-ACC AGC GTG TCC AGG AAG; human β-actin forward 5-CGC GAG AAG ATG ACC CAG AT, human β-actin reverse 5-GAT AGC ACA GCC TGG ATA GCA AC; mouse Pten forward 5- GTT TAC CGG CAG CAT CAA AT, mouse Pten reverse 5- TGG CAG GTA GAA GGC AAC TC; mouse β-actin forward 5- CTT CTA CAA TGA GCT GCG TGT G, and mouse β-actin reverse 5- TGG CAG GTC TCA AAC ATG ATC TGG. Human primers were confirmed not to amplify mouse sequences. Human Pten primers were designed to prevent amplification of the Pten pseudogene on chromosome 9p21.37

Flow cytometry
Intracellular Pten staining was performed on formaldehyde-fixed, methanol-permeabilized cells38 using primary Pten (Y184) antibody (1:100 dilution; Abcam) and secondary goat anti-rabbit AF647 antibody (1:200 dilution; Invitrogen). Intracellular human p16INK4A staining was performed according to the manufacturer’s instructions (p16–fluorescein isothiocyanate kit; BD Biosciences). Routine phenotyping of mouse leukemias used the following antibodies: CD8α-alkyllysocyanin (53-6.7; Biolegend); CD4–phycoerythrin-Cy5.5 (L3T4; eBioscience); CD11b-biotin (M1/70; eBioscience); Gr1-biotin (RB6-SC5; eBioscience); and phycocyanin-conjugated streptavidin (catalog no. 12-4317-87; eBioscience). Acquisition was performed on a FACS Calibur (BD Biosciences) and data were analyzed using FlowJo software (TreeStar).

Histopathology
Mouse tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned for hematoxylin and eosin (H&E) staining. Quantitative assessment of tumor burden was performed by automated image analysis of low-power photomicrographs using QuantityOne software (Bio-Rad). Three histlogic fields were imaged per animal. Image thresholds were set by operators blinded to treatment groups to prevent observer bias. Statistical analysis was performed using GraphPad Prism 5 software.

Results
Generation of primary murine leukemias with activated NOTCH1 retrovirus
Murine bone marrow retroviral transduction/transplantation using activated forms of NOTCH1 is a well-established model for the study of T-ALL.3 Retroviral expression of the NOTCH1 intracellular domain (ICN), which mimics polypeptides derived from the classic but rare cis translocation, produces lethal T-ALL–like disease in mice with high penetrance and short latency. Similarly, HD/PEST mutations, which occur in more than 50% of human T-ALLs, also produce disease in mice albeit with prolonged latency and reduced penetrance compared with ICN, presumably due to weaker activation of signaling.30 For the present study, we elected to generate murine leukemias using NOTCH1 retroviruses with the frequently observed HD and PEST mutations to model human disease more closely. The L1601P–PEST mutant contains a relatively common HD mutation, L1601P, that causes receptor subunit dissociation and subsequent ligand-independent activation,5 in cis with a frameshift mutation that creates a premature termination codon and truncation of the C-terminal negative regulatory PEST domain (ΔPEST). Importantly, the L1601P–ΔPEST allele is leukemogenic in mouse models on wild-type background,30 and is highly similar to HD/PEST mutations present in the Pten-null, GSI-resistant human cell lines, MOLT 3 and P382.20 Of note, activity of the L1601P–ΔPEST construct is blocked by γ-secretase inhibitors (GSIs) because, although subunit dissociation occurs, the transmembrane subunit cannot be cleaved, and thus the intracellular domain remains tethered to the plasma membrane and transcription of downstream target genes does not occur.

To begin address the effects of Pten loss in the murine NOTCH1 leukemia model, we performed retroviral transduction/transplantation using marrow from conditional Pten KO donor mice. We selected a loxP-flanked conditional Pten allele33 in hopes of inducing Cre-mediated deletion in established leukemia cells; however, the interferon-inducible Mx1-Cre driver allele27 was sufficiently active after 5-fluorouracil (5-FU) treatment such that Pten was deleted in more than 50% of marrow progenitors before retroviral transduction (supplemental Figure 1A-B, available on the Blood website; see the Supplemental Materials link at the top of the online article). Consequently, wild-type and Ptenfl/− Mx1-Cre bone marrow (presumably containing a mixture of Ptenfl/fl, Ptenfl/−, and Pten−/− genotypes) was transduced with L1601P–ΔPEST retrovirus, and 45 000 GFP+ cells were transplanted into sublethally irradiated recipients.

NOTCH1 leukemias generated on a Pten-null background exhibit increased penetrance and shortened latency
We observed mice that received a transplant of L1601P–ΔPEST–transduced, Ptenfl/fl, fl/−, −/− marrow succumb to aggressive, disseminated T-ALL disease with 100% penetrance and median latency of 35 days (Figure 1A). All tumors were GFP+ and Pten protein–negative with complete depletion at the Pten locus (Figure 1B and supplemental Figure 1C). Although marrow progenitors with intact Pten were clearly present at the time of retroviral transduction, they are likely “outcompeted” by Pten-null cells during leukemogenic evolution. In contrast, mice that received a transplant of L1601P–ΔPEST–transduced, Ptenfl/fl, fl/−, −/− marrow succumb to aggressive, disseminated T-ALL disease with 100% penetrance and median latency of 35 days (Figure 1A). All tumors were GFP+ and Pten protein–negative with complete depletion at the Pten locus (Figure 1B and supplemental Figure 1C). Although marrow progenitors with intact Pten were clearly present at the time of retroviral transduction, they are likely “outcompeted” by Pten-null cells during leukemogenic evolution. In contrast, mice that received a transplant of L1601P–ΔPEST–transduced, wild-type background marrow developed T-ALL with significantly reduced penetrance (66%, 4 of 6 at 285 days after transplantation) and prolonged latency (median, 81.5 days; P = .001, Figure 1A). Notably, tumors derived on Pten-null and wild-type backgrounds had similar immunophenotype, disease extent/organ distribution, and histology (Figure 1C-D, and supplemental Figure 1D). Consistent with prior studies, we also observed mice that received a transplant of empty vector–transduced, conditional Pten KO marrow (likely also containing Ptenfl/fl, Ptenfl/−, and Pten−/− subsets) developed spontaneous Pten-null disease with 100% penetrance (Figure 1A) including isolated thymic lymphoma (n = 2) and disseminated biphenotypic acute T/myeloid leukemia/lymphoma (n = 2; supplemental Figure 2). Of note, these occurred with significantly longer latency (median, 153.5 days) than disease induced by transduction with
NOTCH1 leukemias generated on a Pten-null background are multiclonal

We also performed TCRβ rearrangement and proviral integration site analysis on primary leukemias from individual mice to gain insight into the clonal diversity of tumors generated on the various genetic backgrounds. Using PCR amplification of genomic DNA to detect TCRβ rearrangements and proviral integration sites, we detected single dominant clones from spontaneous Pten-null thymic lymphomas (without L1601P-PEST) and L1601P-PEST acute T-cell leukemias induced on wild-type background. In contrast, we observed multiple clones from individual L1601P-PEST leukemias on both Pten-null and Pten, Ink4a/Arf double-null backgrounds (Figure 1E). We also observed mice that received a transplant of L1601P-PEST–transduced, Ink4a/Arfnull Mx1-Cre marrow (presumably containing a mixture of Ink4a/Arfnull, Ink4a/Arf+/–, and Ink4a/Arf+/+ genotypes) developed monoclonal tumors (2 of 5 mice died of T-cell leukemia; 3 of 5 died of nonhematologic malignancies; data not shown). Thus, we have observed L1601P-PEST to induce monoclonal disease only on Pten-null backgrounds (with or without Ink4a/Arf) as opposed to monoclonal disease on Pten wild-type backgrounds (with or without Ink4a/Arf). In combination with our observation that L1601P-PEST disease is accelerated on the Pten-null compared with Pten wild-type background (with or without Ink4a/Arf; Figure 1A), these findings suggest that Pten loss may cooperate with activated NOTCH1 in leukemia induction. One important caveat to this conclusion, however, is that Pten loss is known to promote cycling of hematopoietic progenitors, which could increase their retroviral transduction efficiency and contribute to disease acceleration and multiclonality. This issue is considered further in “Discussion.”

Primary murine NOTCH1 leukemia cells lacking Pten remain sensitive to GSI

Consistent with the prior study linking PTEN loss with resistance to Notch inhibition, we observed striking correlation between PTEN loss and GSI resistance among human T-ALL cell lines (supplemental Table 1 and supplemental Figure 3). To determine
whether this association is valid in primary T-cell leukemias derived on a defined genetic background, we treated freshly explanted L1601P-H9004 PEST leukemia cells from both wild-type and Pten-null backgrounds with GSI in vitro for 3 days and measured proliferation, cell size, and apoptosis. Although we noted variation among individual tumors in the degree of response to GSI, both wild-type and Pten-null tumors were GSI sensitive, showing substantially decreased proliferation (Figure 2A and supplemental Figure 4) and reduced cell size (Figure 2B). Furthermore, quantitative comparison of GSI response failed to demonstrate any robust association between Pten loss and even partial GSI resistance (Figure 2A-B). We observed only minimal apoptosis (subG1 fraction) for both wild-type and Pten-null tumors within the 3-day GSI treatment period (supplemental Figure 4).

Interestingly, we also observed a few instances where L1601P-PEST leukemia cells had lost Pten expression spontaneously after either extended culture in vitro (Figure 2C) or serial transplantation in vivo (supplemental Figure 4). In each of these cases, the leukemia cells remained responsive to GSI treatment despite Pten loss. Although the in vitro–cultured cells were ostensibly less GSI sensitive after Pten loss (2.8-fold response at day 29 versus 5.6-fold response at day 0), they also showed a 2.3-fold increase in proliferation without GSI treatment, suggesting rigorous selection for subclones that presumably have accumulated multiple genetic hits in addition to Pten loss. Thus, although Pten loss cannot be excluded as a potential contributing factor, these results clearly demonstrate that Pten loss is not sufficient to confer Notch independence in this genetically defined primary leukemia model.

Primary murine NOTCH1 leukemia cells lacking both Pten and Ink4a/Arf are also sensitive to GSI

Loss of cyclin-dependent kinase inhibitors p16INK4A and p14ARF is perhaps the most frequent genetic alteration in human T-ALL, either by chromosomal deletion or epigenetic silencing. Given that (1) all identified GSI-resistant human T-ALL cell lines are also null for p16INK4A/p14ARF (2) these proteins play a crucial role in the control of cell cycle especially at the G1-S checkpoint, and (3) G1/S cell-cycle arrest is a characteristic response to GSI among sensitive cells, we considered the possibility that GSI resistance may require loss of both Pten and Ink4a/Arf. To address this issue, we generated primary L1601P-PEST leukemias on a Pten, Ink4a/Arf double-null background and assessed GSI response. Again, leaky Mx1-Cre expression presumably
during 5-FU treatment allowed deletion of Pten and Ink4a/Arf in some marrow progenitors that gave rise to all resulting leukemias (Figure 3A, supplemental Figure 6). Despite some tumor-to-tumor variability, leukemias were on the whole sensitive to GSI (Figure 3B) with no discernable degree of resistance conferred by coordinate loss of Pten and Ink4a/Arf (Figure 2A-B). Assessment of subG1 fraction revealed minimal apoptosis after the 3-day GSI treatment period (Figure 3B) similar to that noted for wild-type and Pten-null leukemias (supplemental Figure 4).

To validate these findings in vivo, tumor cells from a single Pten, Ink4a/Arf double-null L1601P-ΔPEST primary leukemia were transplanted into secondary recipients by tail vein injection and engrafted animals subsequently treated with GSI. We allowed a 3-day engraftment period prior to initiation of GSI therapy, as pilot experiments showed secondary recipients all died by 7 days after transplantation (data not shown). All animals were sacrificed on day 3 after initiation of GSI treatment (day 6 after transplantation), and liver parenchyma was assessed histologically for tumor burden. Tumor cells also engrafted bone marrow and spleen, but quantitation of liver involvement by automated image analysis was more robust. Consistent with in vitro findings, we observed significantly fewer lymphoblasts in livers of GSI-treated mice versus control (Figure 3C; P < .0001). Thus, both in vitro and in vivo assays demonstrate that primary mouse NOTCH1 leukemia cells remain sensitive to GSI treatment despite loss of both Pten and Ink4a/Arf tumor suppressors.

Primary human T-ALL cells show no correlation between PTEN loss and Notch independence/GSI resistance

Although mouse models serve an important role in cancer research, it is of growing concern that there may be critical differences between mice and humans in terms of events required for cellular transformation and response to therapy. Our observation of GSI sensitivity among murine NOTCH1 leukemia cells regardless of Pten and Ink4a/Arf status differs from the situation noted in human cell lines20 (supplemental Table 1 and supplemental Figure 3). We felt it was critical therefore to investigate whether PTEN loss conferred Notch independence/GSI resistance using primary human T-ALL samples.

To this end, we assessed a total of 13 patient samples, including 7 cases where lymphoblasts came directly from the patient and 6 cases that were expanded first as xenografts in NOD-SCID/IL2γ−/− (NSG) mice. All patient samples were obtained at initial diagnosis. We cultured human lymphoblasts in vitro on either M5S/MM5-DL1 feeders26 or immobilized DL1 ligand27 for 1 to 6 days prior to initiation of GSI treatment. Cultures were then exposed to GSI versus DMSO vehicle for a 4-day period and subsequently assayed for proliferation, cell size, and apoptosis (Figure 4 and supplemental Figure 7). The 13 cases included 7 PTEN-positive (6 of which were NOTCH1 mutated) and 6 PTEN-negative (4 of which were NOTCH1 mutated) (Table 1). We found 11 of these 13 cases to be GSI sensitive (6 PTEN positive, 5 PTEN negative) as illustrated by decreased proliferation and reduction in cell size (Figure 4 and supplemental Figure 7). We found only 2 cases to be GSI resistant, including 1 PTEN positive and 1 PTEN negative (Figure 5). As with the primary mouse leukemias, we observed only minimal apoptosis within the 4-day GSI treatment period. In these results, we observed no apparent bias to the particular method of culture used for in vitro assay or whether the cells had come directly from the patient versus passaged through NSG mice beforehand (Table 1). Of note, all of the cases were negative for p16INK4A and p14ARF mRNA by reverse-transcription (RT)–PCR and/or protein by flow cytometry (supplemental Figure 8). Thus, in keeping with the mouse results,
we find no evidence for correlation between PTEN loss and GSI resistance in primary human T-ALL cells.

One caveat to note regarding the in vitro culture system used here is that the majority of cases required some form of Delta1 ligand stimulation, whether expressed on the surface of stromal feeders or immobilized on plastic, to “nurture” the cells through the acute stress of transition to in vitro culture. As such, the addition of GSI to assess dependence on Notch signaling essentially withdraws an important aspect of the culture system itself. An alternative approach to assess Notch dependence could have been to plate the cells on MS5 versus MS5-DL1 feeders, or similarly, bare plastic versus immobilized DL1 ligand; however, neither of these methods adequately model withdrawal of NOTCH1 signaling because certain HD mutations presumably confer ligand-independent signaling.5 Moreover, addition of GSI to the cultures is arguably the most direct way to address the particular issue of GSI resistance.

Discussion

In the current study, we have examined the effect of combined NOTCH1 activation and Pten loss in T-cell leukemia induction and observed tumors to occur with shorter latency and exhibit multiclonality, whereas tumors provided with either NOTCH1 activation or Pten loss alone were observed to occur with longer latency and were typically monoclonal or oligoclonal. These observations suggest NOTCH1 activation and Pten loss function cooperatively in the leukemogenic transformation process; however, because our experimental model used retroviral transduction to deliver activated NOTCH1 into hematopoietic progenitors, we cannot exclude the possibility that enhanced retroviral transduction efficiency in cycling Pten-null progenitors33 may contribute to the accelerated disease/increased clonality we have observed. Of note, we did not observe a similar disease acceleration/increased clonality in leukemias derived on the Ink4a/Arf-null background, which is also likely to enhance cycling of hematopoietic progenitors.41,42 Because of this potential limitation, however, further studies will be required to address the issue of oncogenic collaboration in a more definitive manner.

We also addressed whether indeed PTEN loss confers resistance to Notch inhibition as has been suggested by cell line studies.20 Using primary human and murine T-cell leukemia samples, we observed no correlation to exist between loss of PTEN and GSI resistance. Of note, Cullion et al observed murine Tal1 leukemias lacking Pten also to remain sensitive to GSI treatment.43 These findings raise the question as to what complement of secondary genetic alterations can indeed relieve T-cell leukemias of their addiction to Notch signaling. It was proposed recently that FBW7 mutation may contribute to GSI resistance by reducing c-Myc protein turnover.35 Although there was only a single case among our assayed primary human T-ALL samples that carried an FBW7 mutation (KER, Table 1), we found this case to be sensitive to GSI (supplemental Figure 7). This particular sample is PTEN wild type, however, and because FBW7 mutations are notably present in several identified PTEN-null, GSI-resistant human cell lines, it remains an open question as to whether the combination of Pten-null and FBW7 mutation may lead to GSI resistance. FBW7 mutation likely cannot be a requisite feature of GSI resistance, however, because FBW7 mutations are lacking from the 2 GSI-resistant primary human cases reported here and also from several GSI-resistant cell lines.34,35
Another opportunity to examine the issue of Notch independence in T-ALL is presented by mouse models in which Notch1 mutations do not occur. Although the majority do show frequent Notch1 mutations, T-cell leukemias arising in mice with activated β-catenin in thymocytes and deletion of Pten in hemangio-blastic precursors are notable exceptions lacking Notch1 mutations. Leukemias in both models showed consistent up-regulation of c-Myc and activation of β-catenin, and have led to the intriguing hypothesis that the combination of Wnt/β-catenin, c-Myc, and PI3K activation can functionally replace the need for Notch1 signaling. In this regard, it is notable that NOTCH1 directly induces c-Myc expression and may also repress FBW7 via PI3K activation. Because Notch inhibitors will find their greatest clinical utility in patients with refractory/relapsed disease, determining the possibly including but not necessarily limited to PI3K/Akt activation.

The high frequency of NOTCH1 mutation in human T-ALL has sparked considerable interest in Notch signaling inhibitors as targeted therapy. Although early clinical studies have identified goblet cell hyperplasia-associated secretory diarrhea as a dose-limiting toxicity, both intermittent dosing strategies and coadministration of glucocorticoids may help to ameliorate these gastrointestinal side effects yet allow a therapeutic window to be achieved. We observed GSI resistance to occur in only a small fraction of primary human samples (2/13 assayed; 15%), and we noted 4 (44%) of 9 wild-type leukemias to undergo massive growth arrest, possibly including but not necessarily limited to PI3K/Akt activation.

Table 1. Summary of results for primary human T-ALL cells: no correlation between GSI response and PTEN status

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<th>PTEN mutation</th>
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<td>M34</td>
<td>WT</td>
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<td>M53</td>
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<td>M69</td>
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<td>WT</td>
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<td>1615 Del (QM) Ins (L)</td>
<td>2515 RVP*</td>
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<td>Resistant</td>
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<td>WT</td>
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<td>L1601P</td>
<td>WT</td>
<td>WT</td>
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</table>

NOTCH1, PTEN, and FBW7 status in 20 human T-ALL samples included in this study. GSI response was assessed in 13 of these using lymphoblasts directly from patients (fresh) and/or after expansion as xenografts in NSG mice (NSG). The 3 samples assessed in both situations yielded consistent results. PTEN protein was assessed by intracellular flow cytometry. Observed mutation frequencies among 18 unselected cases are similar to previous reports (D115 and D135 were specifically selected for NOTCH1 and PTEN mutation).

WT indicates wild type; and ND, not determined.

*Premature stop codon.
incidence of GSI resistance and relevance of PTEN loss in this setting certainly warrants further study.

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Authorship

Contribution: H.M. and S.G. designed, performed, and analyzed experiments; F.A. and F.P. provided methodological expertise for expansion of human T-ALL samples; X.G. and Q.L. generated conditional knockout animals; F.A., A.L.G., L.H.M., K.R.S., and F.P. provided genetically characterized patient samples and discussed results; M.J.Y. and A.P.W. conceived and supervised the study; and H.M. and A.P.W. wrote the paper.

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References


Acute T-cell leukemias remain dependent on Notch signaling despite PTEN and INK4A/ARF loss

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