REVIEW ARTICLE

# The relevance of PTEN-AKT in relation to NOTCH1-directed treatment strategies in T-cell acute lymphoblastic leukemia

Rui D. Mendes,[1] Kirsten Canté-Barrett,[1] Rob Pieters,[1,2] and Jules P.P. Meijerink[1,2]

[1]Department of Pediatric Oncology/Hematology, Erasmus MC Rotterdam-Sophia Children's Hospital, Rotterdam, The Netherlands; and [2]Princess Máxima Center for Pediatric Oncology, Utrecht, The Netherlands

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## ABSTRACT

The tumor suppressor phosphatase and tensin homolog (PTEN) negatively regulates phosphatidylinositol 3-kinase (PI3K)-AKT signaling and is often inactivated by mutations (including deletions) in a variety of cancer types, including T-cell acute lymphoblastic leukemia. Here we review mutation-associated mechanisms that inactivate PTEN together with other molecular mechanisms that activate AKT and contribute to T-cell leukemogenesis. In addition, we discuss how *Pten* mutations in mouse models affect the efficacy of gamma-secretase inhibitors to block NOTCH1 signaling through activation of AKT. Based on these models and on observations in primary diagnostic samples from patients with T-cell acute lymphoblastic leukemia, we speculate that PTEN-deficient cells employ an intrinsic homeostatic mechanism in which PI3K-AKT signaling is dampened over time. As a result of this reduced PI3K-AKT signaling, the level of AKT activation may be insufficient to compensate for NOTCH1 inhibition, resulting in responsiveness to gamma-secretase inhibitors. On the other hand, *de novo* acquired PTEN-inactivating events in NOTCH1-dependent leukemia could result in temporary, strong activation of PI3K-AKT signaling, increased glycolysis and glutaminolysis, and consequently gamma-secretase inhibitor resistance. Due to the central role of PTEN-AKT signaling and in the resistance to NOTCH1 inhibition, AKT inhibitors may be a promising addition to current treatment protocols for T-cell acute lymphoblastic leukemia.

**Correspondence:**
j.meijerink@erasmusmc.nl

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## T-cell acute lymphoblastic leukemia

T-cell acute lymphoblastic leukemia (T-ALL) is a cancer of developing T cells in the thymus. T-ALL is characterized by chromosomal rearrangements. These rearrangements can lead to the aberrant activation of oncogenic transcription factors by placing their genes under the control of promoters and/or enhancers of T-cell receptor genes, the *BCL11B* gene, or other genes; occasionally, these rearrangements can give rise to oncogenic fusion proteins. The activated oncogenic transcription factors include *TAL1* and *LMO2* (and related family members), *TLX1*, *TLX3*, *NKX2-1*, *HOXA*, and *MEF2C*; in addition, certain oncogenic fusion proteins can directly activate the *HOXA* or *MEF2C* genes.[1,2] Oncogenic proteins facilitate the developmental arrest of pre-leukemic immature T cells. We previously proposed that these chromosomal rearrangements should be classified as type A aberrations, as they are generally considered to be the driving oncogenic event associated with unique expression profiles.[2] Based upon their gene expression signatures, T-ALL can be classified into the following four major subtypes: ETP-ALL, TLX, proliferative, and TALLMO.[3-5]

Maturation arrest induces a pre-leukemic condition in which additional mutations can give rise to T-ALL.[1,2] These secondary mutations are not necessarily clonal events and are often selected during disease progression or post-treatment

relapse.[6,7] We therefore proposed that these mutations should be classified as type B aberrations.[2] Type B mutations are prevalent among all T-ALL subtypes and affect a wide variety of cellular processes, including survival and proliferation, cell cycle progression, and epigenetic events. Type B mutations often affect signal transduction pathways, including the NOTCH1, IL7R-JAK-STAT, RAS-MEK-ERK, and PTEN-PI3K-AKT pathways. A growing body of evidence suggests that some of these signaling pathways are preferentially mutated in specific T-ALL subtypes, presumably due to the fact that developing T cells are dependent on these pathways in specific stages. For example, mutations in IL7 receptor (IL7R) and the downstream molecules JAK or RAS are prevalent among TLX and ETP-ALL patients.[8-10] Although new therapeutic strategies that target oncogenic transcription factor complexes are emerging,[11] several compounds that selectively inhibit altered signaling pathways are currently available. Thus, inhibiting signaling proteins such as NOTCH, IL7R, RAS and/or AKT may provide a promising new therapeutic approach for T-ALL.

In this review, we describe the role of PTEN as a tumor suppressor and we discuss various PTEN-inactivating mechanisms observed in different human cancers and T-ALL. Besides PTEN inactivation, we describe other mechanisms that contribute to AKT activation and leukemogenesis. Finally, we discuss PTEN-AKT signaling in relation to future NOTCH1-directed therapies and provide a rationale for the use of AKT inhibitors in addition to current treatment protocols.

## The PTEN tumor suppressor

Mutations in the tumor suppressor gene *PTEN* (phosphatase and tensin homolog), which is located on chromosomal band 10q23, are very common in a wide range of cancers.[12,13] The *PTEN* gene contains nine exons, and the encoded protein includes an N-terminal phosphatase domain, a central C2 lipid membrane-binding domain, and a C-terminal tail domain (Figure 1). PTEN is a phosphatase that dephosphorylates $PIP_3$ [phosphatidylinositol (3,4,5)-triphosphate] to produce $PIP_2$ [phosphatidylinositol (4,5)-bisphosphate], thereby opposing the function of PI3K (phosphatidylinositol 3-kinase). PI3K converts $PIP_2$ into $PIP_3$, which in turn activates key downstream kinases, including PDK1 and AKT (Figure 2). Thus, PTEN is an important negative regulator of PI3K-AKT signaling. Because AKT plays key roles in cellular metabolism, proliferation and survival, inactivation of PTEN by genetic aberrations drives survival and uncontrolled proliferation, ultimately leading to cancer.[14] A recent study identified an alternate translation initiation site located upstream of the coding region of canonical *PTEN* that generates a larger form of PTEN.[15] This isoform is known as PTENα and is described to be involved in mitochondrial energy metabolism.[15]

## PTEN aberrations in cancer

Heterozygous germline mutations in *PTEN* were identified initially in 60-80% of patients with a group of rare syndromes including Cowden syndrome, Bannayan-Riley-Ruvalcaba syndrome, and PTEN-related Proteus syndrome; these disorders are known collectively as PTEN hamartoma tumor syndrome (PHTS).[16] With respect to sporadic (i.e., non-hereditary) tumors, heterozygous *PTEN* mutations occur in 50-80% of prostate, glioblastoma, and endometrial cancers and 30-50% of lung, colon, and breast cancers.[17] Loss of both functional *PTEN* alleles is common among patients with prostate or breast cancer, as well as

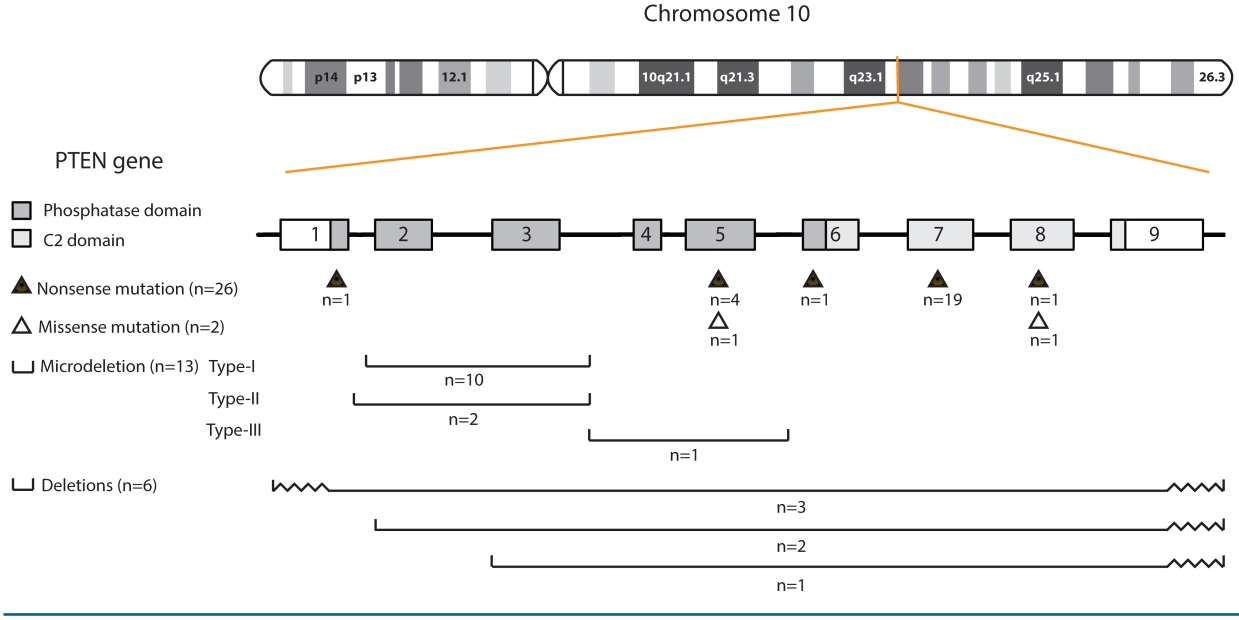


**Figure 1. Schematic representation of the human *PTEN* gene located on chromosome 10q23.** The *PTEN* gene contains nine exons, and the PTEN protein contains several functional domains, including a phosphatase domain (dark gray) and a C2 lipid-binding domain (light gray). The positions of nonsense insertion and deletion mutations are indicated by closed triangles, and missense mutations are indicated by open triangles. Microdeletions and deletions in the *PTEN* gene are shown below the exons. The number of patients with each mutation/deletion in our cohort of T-ALL patients is indicated.[21, 35]

among those with melanoma or glioblastoma.[18] The majority of these aberrations are caused by point mutations, small insertions, or deletions, all of which can occur throughout the entire *PTEN* gene. At the transcriptional and post-transcriptional levels, PTEN inactivation can occur *via* promoter methylation and through the expression of *PTEN*-directed microRNA.[19] PTEN activity is also regulated at the post-translational level: phosphorylation, ubiquitination, oxidation, and acetylation can regulate the phosphatase activity, subcellular localization, and degradation of PTEN.[17] Defects in any of these processes may explain the absence of functional PTEN in cancer patients who apparently lack genetic aberrations in *PTEN*.[20-22] Several ALL cases have been identified in which high lev-

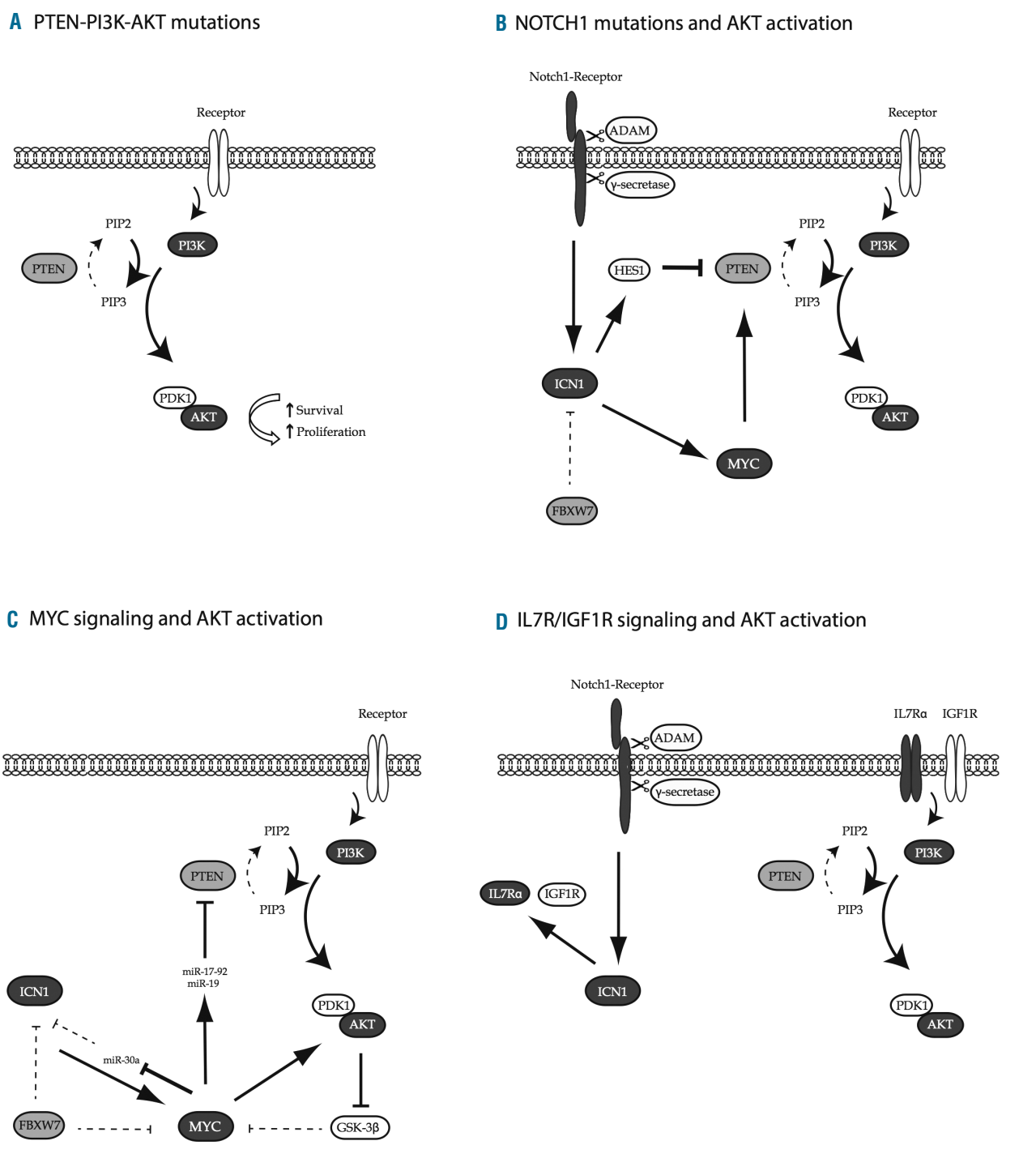


**Figure 2. Schematic overview of the upstream and downstream effectors of PTEN and associated molecular mechanisms that can activate AKT and lead to GSI resistance.** (A) PTEN-PI3K-AKT mutations. (B) NOTCH mutations and AKT activation. (C) MYC signaling and AKT activation. (D) IL7R/IGF1R signaling and AKT activation. The molecules with activating and inactivating mutations are indicated in dark gray and light gray, respectively. Dashed lines/arrows represent processes that contribute to cellular GSI sensitivity, and that are frequently inactivated by inactivating mutations/rearrangements in *PTEN* or *FBXW7*. Solid lines/arrows represent GSI resistance mechanisms.

els of inactive PTEN are accompanied by an active PI3K-AKT pathway.[23,24] Although the majority of prevalent pathogenic mechanisms affect the loss of one or both *PTEN* alleles, subtle changes in PTEN protein levels can have a powerful effect on cancer susceptibility and/or tumor progression, as exemplified by the *Pten* hypomorphic mouse model.[25] Thus, the level of functional PTEN affects tumor susceptibility, and PTEN function can be compromised at the DNA, mRNA, and/or protein levels.

## PTEN aberrations in T-cell acute lymphoblastic leukemia

*PTEN* deletions and mutations were initially identified in cell lines.[26,27] Restoring PTEN levels in these cell lines decreased cell size and induced apoptosis by suppressing the PI3K-AKT pathway.[28] Studies by others[29-34] and our group[21,35] revealed aberrations in the PTEN-PI3K-AKT pathway in approximately 23% of primary samples obtained from pediatric T-ALL patients. With respect to T-ALL subtypes, we have shown that *PTEN* aberrations are strongly associated with TAL- or LMO-rearranged leukemia in children[21] and the same was observed in adult T-ALL cohorts.[36] The vast majority of *PTEN* aberrations are nonsense mutations in exon 7 (which truncate the C-terminal domain) and deletions that affect nearly the entire locus (Figure 1). Although truncated PTEN proteins that lack the lipid-binding C-terminal domain retain their phosphatase activity, they are highly unstable and are degraded rapidly.[37] In mice, truncated PTEN leads to decreased genomic stability and the development of multiple cancers.[38] Recently, we reported that approximately 8% of T-ALL patients have a RAG-mediated microdeletion in the phosphatase domain that disrupts the reading frame (Figure 1).[35] In addition, mutations have been identified in *PI3K* and *AKT*; specifically, 9% of pediatric T-ALL patients have a mutation in either the catalytic (*PIK3CA*) or regulatory (*PIK3R1*) subunit of *PI3K*, and 2% of patients have a mutation in *AKT* itself (Figure 2A).[21,30] Many T-ALL patients with a heterozygous *PTEN* mutation also acquire a deletion[21] or microdeletion[35] in their remaining wild-type allele in leukemic subclones[29] that may give rise to relapse. This phenomenon was demonstrated functionally by Clappier *et al.* who used an elegant human T-ALL xenograft transplantation model in mice and found the selection and preferential outgrowth of PTEN-inactivated leukemic cells.[39] In line with this, heterozygous *Pten* knockout mice develop T-cell leukemia in which the remaining wild-type allele is frequently deleted.[40-42] Other leukemogenic mechanisms that can inactivate PTEN at the protein level are the increased expression of casein kinase 2 (CK2) and the production of reactive oxygen species (ROS) that stabilize inactive forms of PTEN proteins and lead to impaired phosphatase activity.[23,43] Collectively, these findings indicate the existence of ongoing pathogenic pressure to inactivate both *PTEN* alleles during disease progression, and the resulting loss of PTEN activity in turn activates the PI3K-AKT pathway.

## Clinical implications

We have reported that aberrations in PTEN represent a significant, independent risk factor for relapse in T-ALL patients treated using either the Dutch Childhood Oncology Group or German Cooperative Study Group for Childhood ALL protocol.[21,35] Similar results were reported for other cohorts of pediatric T-ALL patients treated using other protocols.[32,33] In the Berlin-Frankfurt-Munster study, the presence of NOTCH1-activating mutations in addition to PTEN-inactivating mutations predicts for good outcome similar to that of patients harboring NOTCH1-activating mutations only,[32] suggesting that NOTCH1-mutations can antagonize the unfavorable effect of PTEN aberrations. In a French Group for Research in Adult ALL study of T-ALL patients, those with aberrations in *RAS* and/or *PTEN* had a significantly worse outcome compared to patients without such mutations.[36] This was not confirmed in the MRC UKALL2003 trial for pediatric T-ALL; *RAS* and/or *PTEN* aberrations also did not change the favorable outcome of patients with *NOTCH1/FBWX7* mutations.[44] Taken together, these findings suggest that PTEN aberrations may represent a general, poor prognostic factor in T-ALL.

## NOTCH1 mutations lead to activation of AKT

More than 65% of T-ALL patients have aberrant activation of the NOTCH1 pathway due to mutations in either the *NOTCH1* gene itself or *FBXW7*, which encodes E3-ubiquitin ligase[45,46] Thus, the NOTCH1 pathway may be an ideal target for therapeutic intervention. Furthermore, NOTCH1-directed therapies are clinically important, as they can also boost the cellular response to steroids.[47,48] Gamma-secretase inhibitors (GSI), which inhibit the presenilin gamma-secretase complex, block the cleavage of NOTCH1 at its S3 site; this cleavage step is required to release the active, intracellular NOTCH1 domain (ICN1) upon ligand binding (Figure 2B). Several groups have applied GSI to cell lines derived from T-ALL patients with *NOTCH1*-activating mutations; although GSI treatment initially induces cell cycle arrest, the majority of cell lines adapt and ultimately stop responding to the treatment (i.e., develop GSI resistance).[45,49] Nevertheless, GSI treatment effectively blocks gamma-secretase activity, resulting in reduced intracellular levels of the ICN1 domain and reduced expression of NOTCH1's target genes.[29] GSI resistance is, therefore, caused by other mechanisms that circumvent NOTCH1 inhibition.[50,51] Consistent with this notion, Palomero and co-workers found that decreased PTEN levels in cell lines are correlated with GSI resistance, and GSI-resistant lines have increased levels of activated AKT.[29] Restoring the expression of functional PTEN in these GSI-resistant lines restored a GSI sensitivity response, whereas constitutively activated AKT or using shRNA to knock down PTEN expression provoked GSI resistance in a GSI-responsive line.[29] This seminal study identified two important NOTCH1 downstream targets that regulate *PTEN* expression: HES1 and MYC. HES1 is a robust transcriptional repressor, whereas MYC is a weak transcriptional activator. Because the negative effect of HES1 prevails over the positive effect of MYC, *PTEN* expression is suppressed (Figure 2B).[29]

However, the resistance of leukemic cells to GSI resulted in disappointing results upon testing the GSI inhibitor MK-0752 in a clinical trial (DFCI-04-390).[52] This trial was unsuccessful due to the compound's limited efficacy in leukemic cells and severe gastrointestinal toxicity. To overcome these issues, next-generation NOTCH1 inhibitors

with reduced off-target toxicity are currently in development.[53] For example, promising strategies include selectively blocking NOTCH1 using anti-NOTCH1 antibodies[54,55] or chemically modified peptides that block the NOTCH transcriptional complex in the nucleus.[56]

## PTEN is not linked *a priori* to resistance to gamma-secretase inhibitors in human T-cell acute lymphoblastic leukemia

Despite the initial report by Palomero and co-workers,[29] subsequent studies have not confirmed that loss of PTEN activity is intrinsically linked to GSI resistance.[21,57,58] For example, GSI sensitivity was similar between NOTCH1-driven T-cell leukemia cells obtained from wild-type mice and from PTEN knockout mice.[58] However, PTEN deficiency does accelerate the disease progression of NOTCH1-driven leukemia.[58] Using a different *Pten* knockout mouse model (*Pten*$^{flox/flox}$/*Lck-Cre*), Hagenbeek *et al.* found that PTEN-deficient thymocytes were just as sensitive to *in vitro* GSI treatment as were wild-type thymocytes.[57] Moreover, several human T-ALL cell lines with mutant alleles of *PTEN* – different cell lines from those used by Palomero *et al.* – were actually sensitive to GSI.[21] In diagnostic samples from patients with TALLMO, PTEN is frequently inactivated in the absence of NOTCH-activating mutations.[21,32,36,59] Thus, mutations in *PTEN* and *NOTCH1/FBXW7* are frequently independent genetic events and only co-occur in a small number of patients' primary samples. In those patients who harbor both *PTEN* mutations and *NOTCH1/FBXW7* mutations, the *NOTCH1* mutations are usually weakly activating mutations. Because *PTEN* and *NOTCH1* mutations are mostly independent genetic events in primary T-ALL, PTEN-deficient leukemic cells in T-ALL patients likely do not have intrinsic GSI resistance at disease presentation. Perhaps one way that PTEN-deficient T-ALL can be linked to GSI resistance is upon relapse, when NOTCH1-dependent leukemic cells may have lost PTEN activity, possibly due to clonal selection following treatment. However, there is currently no evidence to support this notion.

The question remains, is it possible that immediately following PTEN loss, NOTCH1-dependent T-ALL becomes NOTCH1-independent and develops GSI-resistance? Recently, Adolfo Ferrando's group addressed this intriguing question by generating an elegant mouse model of NOTCH1-induced T-ALL in which the *Pten* gene is deleted only in established tumors.[60] Unlike previous Pten knockout models,[57,58] deletion of *Pten* in this new model conferred strong resistance to dibenzazepine, a potent GSI. In this model,[60] *Pten* loss activated expression of genes involved in cell metabolism, ribosomal RNA processing, and amino acid and nucleotide biosynthesis, genes that are normally suppressed following NOTCH1-inhibiting GSI treatment. Moreover, GSI treatment increased leukemic cells' dependency on autophagy in order to recycle essential metabolites. *Pten* loss also relieved the GSI-instigated block of glycolysis and glutaminolysis, a phenotype that was copied by expressing the constitutively active myrisoylated AKT. Because both the GSI dibenzazepine and the glutaminase inhibitor BPTES [bis-2-(5-phenylacetamido-1,2,4-thiadiazol-2-yl)ethyl sulfide 3] act in a synergistic fashion in inducing anti-leukemic effects, the authors proposed glutaminolysis as a major therapeutic target for treating NOTCH-activated T-ALL.[60]

Another unanswered question remains: why does the loss of *Pten* in an established NOTCH1-driven tumor cause GSI resistance,[60] while NOTCH1-driven tumors that are generated in *Pten* knockout mice remain GSI-sensitive?[57,58] The answer may lie in the ability of cells to adapt to PTEN loss by dampening PI3K-Akt signaling over time to a level that is still of advantage to leukemic cells. Unlike progressive reduction in PI3K-Akt signaling, the loss of PTEN may initially drive the rapid, high activation of Akt, resulting in cell proliferation, survival, and GSI resistance. This hypothesis predicts two consequences of GSI or other NOTCH1-inhibiting treatment. First, T-ALL patients who lack PTEN activity at disease onset may still respond to NOTCH1 inhibition. Second, NOTCH1 inhibition may trigger leukemic cells to acquire mutations such as *PTEN* deletions, which leads to activation of AKT and resistance to NOTCH1 inhibitors. Several key observations provide support for this hypothesis of reduced PI3K-AKT signaling over time in the absence of PTEN. For example, we found no difference in AKT phosphorylation between primary T-ALL patients with aberrant *PTEN* and patients without such mutations,[21] indicating that patients with *PTEN*-defective leukemia may have adapted and reduced PI3K-AKT signaling. Reduced AKT activation may explain why primary *PTEN*-defective T-ALL cells are NOTCH1-dependent and remain GSI-sensitive.[58] Although this hypothesis has not been tested formally, future NOTCH-inhibiting therapies may be more effective when combined with inhibitors of PI3K or AKT. Consistent with this, the PI3K/mTOR dual inhibitor PI-103 resulted in enhanced NOTCH-MYC activity in T-ALL cell lines.[61] Additionally, T-ALL induced by retroviral insertional mutagenesis in wild-type or *RasG12D*-mutant mice demonstrated an initial response to PI3K-inhibitor GDC-0941 treatment.[62] However, this treatment led to the survival and outgrowth of drug-resistant clones with active PI3K-AKT signaling that frequently had reduced Notch1 signaling.[62] To avoid resistance when combined with NOTCH1 inhibitors, the authors propose a sequential treatment using a NOTCH1 inhibitor at diagnosis to eliminate NOTCH1 mutant clones followed by PI3K/AKT inhibitor treatment.[62]

## Other mechanisms that can activate AKT and lead to resistance to gamma-secretase inhibitors

Eighty-five percent of T-ALL patients have an activated AKT pathway accompanied by increased phosphorylation of AKT and its downstream targets GSK-3β and FOXO3a.[23] Notably, this percentage is higher than the frequency of PTEN aberrations (present in 23% of patients) and, therefore, has to be explained by the activation of AKT through other mechanisms.

MYC may provide an alternative mechanism to activate AKT either directly or indirectly (e.g. MYC activates the expression of mir-17-92 and mir-19, which target *PTEN* mRNA)[63-65] (Figure 2C). Using an inducible MYC-dependent zebrafish T-ALL model, Gutierrez *et al.* found that established tumors regressed when MYC expression was turned off. This effect was circumvented by activating PI3K-AKT signaling,[66] showing that AKT activation is an important downstream effector of MYC which may drive GSI resistance. Moreover, the *MYC* gene is an important

downstream target of NOTCH1, and T-ALL patients with activating mutations in *NOTCH1* overexpress MYC.[67,68] NOTCH binds a distal enhancer located far downstream of the *MYC* locus.[69,70] This NOTCH-MYC enhancer region (N-Me) is duplicated in approximately 5% of T-ALL patients, acting as a "super-enhancer".[69] In another 6% of adult and childhood T-ALL patients, MYC is ectopically activated due to a *MYC* translocation; importantly, these patients usually do not have *NOTCH1*-activating mutations.[71] MYC may also activate NOTCH1 *via* a positive feedback mechanism, as MYC suppresses the expression of miRNA-30, which targets the 3' untranslated region of *NOTCH1* (Figure 2C).[72] Accordingly, treatment of T-ALL xenografted mice with the bromodomain protein inhibitor JQ1 results in decreased MYC levels and also reverses MYC-induced resistance to GSI.[73,74] Furthermore, in human T-ALL cell lines, GSI-sensitive cells can be converted to being GSI-resistant by the ectopic expression of MYC.[68,75] Under normal conditions, MYC is phosphorylated by the kinase GSK-3β; phosphorylated MYC is then subjected to ubiquitination by FBXW7 and proteasome-mediated degradation (Figure 2C).[76,77] Conversely, activated AKT can stabilize MYC protein by phosphorylating – and thereby inactivating – GSK-3β. These findings may explain the observation that MYC and PTEN are reciprocally expressed in T-ALL.[78]

Apart from enhancing cellular resistance to NOTCH1 inhibitors, MYC also enhances leukemia-initiating cell activity and worsens outcome in various mouse models of T-ALL. Mutant *Fbxw7-R465C* mice develop aggressive leukemias that acquire *Notch1* mutations.[79] Myc levels are stabilized in these mice, resulting in the expansion of leukemia cells that have enhanced self-renewal capacity and that express a stem cell-like expression profile.[79] The *Tal1/Lmo2* transgenic mouse model develops spontaneous T-cell tumors that also acquire *Notch1* mutations. Because *Myc* is a Notch target, Notch inhibition led to reduced leukemia-initiating cell activity in these mice.[80] Reducing endogenous Myc levels led to increased survival and reduced numbers of leukemia cells with leukemia-initiating cell potential in both models.[79,81] Overall, these positive feedback loops between NOTCH, MYC, and AKT suggest that inhibitors of MYC or PI3K/AKT may help to prevent resistance to NOTCH1-inhibiting therapies,[82] and also eliminate leukemia-initiating cell activity in T-ALL. Co-targeting the PI3K pathway and MYC remarkably enhanced the elimination of leukemia-initiating cells.[83]

Another AKT activation mechanism is *via* the gene that encodes the IL7 receptor (IL7Ra), which also represents a direct target gene of NOTCH1.[84,85] The *IL7R* gene is mutated in nearly 10% of T-ALL patients. These mutations cause the constitutive activation of STAT5 and AKT,[8,86,87] and can provoke GSI resistance (Figure 2D). For instance, expression of the IL7Ra can overcome the effects of NOTCH1 inhibition on the cell cycle and survival, thereby contributing to resistance.[84] Similar results were obtained by overexpressing *IGF1R*, which encodes insulin-like growth factor 1 receptor and is another NOTCH1 target (Figure 2D).[88] In these cases, too, NOTCH-inhibiting therapies may be more effective when combined with AKT inhibitors. Furthermore, enhanced AKT activity may limit leukemia sensitivity to steroid treatment,[89,90] one of the cornerstone drugs in the treatment of human T-ALL. AKT was shown to directly phosphorylate (S134) and inactivate the steroid receptor NR3C1.[89] Combined steroid treatment with the dual PI3K-mTOR inhibitor BEZ235[91] or the MK2206 AKT inhibitor[89] sensitized AKT-activated leukemic cells to steroid treatment.

## Conclusion

As a potent tumor suppressor, PTEN is considered to be the principal negative regulator of PI3K-AKT signaling. Inactivation of PTEN indirectly activates PI3K-AKT signaling, causing the uncontrolled proliferation of thymocytes, ultimately leading to T-ALL. Regardless of PTEN, AKT can be over-activated by a variety of signaling molecules, including PI3K, AKT, MYC, IL7R and IGF1R (Figure 2). Initial activation of AKT causes resistance to NOTCH1-inhibiting therapies. However, in the long-term, we suggest that AKT signaling may be dampened, thereby restoring responsiveness to NOTCH-inhibiting therapies. Overall, because AKT activation is central to a variety of leukemogenic mechanisms and crucial in the resistance to NOTCH1 inhibition, using AKT inhibitors in current treatment protocols may be a promising strategy to treat NOTCH1-mutated T-ALL.

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