Targeting the phosphatidylinositol 3-kinase/Akt/mechanistic target of rapamycin signaling pathway in B-lineage acute lymphoblastic leukemia: An update

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Despite considerable progress in treatment protocols, B-lineage acute lymphoblastic leukemia (B-ALL) displays a poor prognosis in about 15–20% of pediatric cases and about 60% of adult patients. In addition, life-long irreversible late effects from chemotherapy and radiation therapy, including secondary malignancies, are a growing problem for leukemia survivors. Targeted therapy holds promising perspectives for cancer treatment as it may be more effective and have fewer side effects than conventional therapies. The phosphatidylinositol 3-phosphate kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR) signaling pathway is a key regulatory cascade which controls proliferation, survival and drug-resistance of cancer cells, and it is frequently upregulated in the different subtypes of B-ALL, where it plays important roles in the pathophysiology, maintenance and progression of the disease. Moreover, activation of this signaling cascade portends a poorer prognosis in both pediatric and adult B-ALL patients. Promising preclinical data on PI3K/Akt/mTOR inhibitors have documented their anticancer activity in B-ALL and some of these novel drugs have entered clinical trials as they could lead to a longer event-free survival and reduce therapy-associated toxicity for patients with B-ALL. This review highlights the current status of PI3K/Akt/mTOR inhibitors in B-ALL, with an emphasis on emerging evidence of the superior efficacy of synergistic combinations involving the use of traditional chemotherapeutics or other novel, targeted agents.

KEYWORDS
B-ALL, combination therapy, PI3K/Akt/mTOR signaling, protein kinase inhibitors, targeted therapy

1 | INTRODUCTION

B-lineage acute lymphoblastic leukemia (B-ALL) is a hematological malignancy characterized by the uncontrolled proliferation of B-cell precursors. B-ALL displays a bimodal distribution with approximately 60% of cases occurring in patients younger than 20 years of age and accounting for roughly 80% of pediatric leukemias (Pui, Relling, & Downing, 2004). The second peak occurs around the fifth decade of life, however it accounts for only 20% of leukemias diagnosed in adult patients. At present, 95% of pediatric B-ALL patients reach a complete remission (CR), while the estimated 5-year survival rates...
similar to that of Ph+ B-ALL, however the Bcr-Abl fusion protein is not detected in 10% of childhood B-ALL cases are Ph-like (Tran & Loh, 2016). This finding is relevant as it identifies high-risk B-ALL subtype. It occurs more frequently in children aged ≤5 years old (Pui & Evans, 2006). Depending on the translocation breakpoint in Bcr, different Bcr-Abl isoforms are expressed, which all contain exons 2–11 of the Abl gene, but different lengths of their Bcr component (Deininger, Goldman, & Melo, 2000). The most common Bcr-Abl isoforms are p190 (alternatively named: p185) and p210. While p210 is the hallmark of chronic myelogenous leukemia, p190 occurs in the majority of Ph+ B-ALL. It is intriguing that, very recently, marked differences in the expression of Bcr-Abl isoforms were found (Cutler et al., 2017; Reckel et al., 2017). Besides other aspects, these findings could give important indications regarding differential sensitivity to kinase inhibitors and other targeted agents.

Philadelphia chromosome-like B-ALL (Ph-like B-ALL) is a recently identified high-risk B-ALL subtype. It occurs more frequently in adolescent and adult patients, having been reported in as many as 27% of young adults with B-ALL between 21 and 39 years of age, while only 10% of childhood B-ALL cases are Ph-like (Tran & Loh, 2016). This aggressive B-ALL subtype is characterized by a gene expression profile similar to that of Ph+ B-ALL, however the Bcr-Abl fusion protein is not expressed. Nevertheless, Ph-like B-ALL cells harbor a multitude of genetic alterations that activate several cytokine receptor genes and kinase signaling pathways resulting in a consequent poor prognosis (Ofran & Izraeli, 2017).

Although in childhood B-ALL patients multiagent chemotherapy has had a great success, the same is not true in adults, even with the introduction of tyrosine kinase inhibitors (TKIs) targeting Bcr-Abl kinase, in combination with chemotherapy (Malagola, Papayannidis, & Baccarani, 2016).

Moreover, in refractory or relapsed settings, the outcome of pediatric B-ALL is still poor (Pui & Evans, 2006). Furthermore, the long-term adverse effects of polychemotherapy and radiotherapy increase the risk of morbidity and premature death, especially in children (Ness, Armenian, Kadan-Lottick, & Gurney, 2011). Therefore, the identification of novel targeted therapies for supporting conventional chemotherapy is urgently needed to further improve the outcome of B-ALL in both adult and pediatric patients (Dias, Kenderian, Westin, & Litzow, 2016).

A promising candidate for the development of targeted therapies is the phosphatidylinositol kinase (PI3K)/Akt/mechanistic target of rapamycin (mTOR) pathway. This signaling cascade is involved in a wide array of physiological processes whose deregulation is frequently associated with tumorigenesis, including mRNA translation, apoptosis, autophagy, metabolism, migration, and angiogenesis (Pons-Tostivint, Thibault, & Guillermet-Guibert, 2017; Bahrami et al., 2018).

Overall, this pathway is one of the most frequently mutated and aberrantly activated networks in different types of cancers (Guerrero-Zotano, Mayer, & Arteaga, 2016), including acute leukemias (Bertacchini et al., 2015). For this reason, pharmaceutical companies have developed many selective inhibitors of the key effectors of this complex signaling cascade (Rodon & Tabernero, 2017). However, a growing body of evidence suggests that small molecules targeting PI3K/Akt/mTOR signaling are most effective against cancer cells when used in combination with other therapeutic agents.

Aberrant activity of the PI3K/Akt/mTOR network is commonly detected in adult B-ALL (Gomes et al., 2014), while pathway activation is associated with poor prognosis and chemoresistance in pediatric B-ALL cases (Morishita et al., 2012; Nemes et al., 2013), and high expression of mTOR has been very recently demonstrated to correlate with a poor clinical outcome in both adult and childhood B-ALL (Khanna et al., 2017). Moreover, glycogen synthase kinase-3β (GSK-3β), an Akt downstream target (Hermida, Dinesh Kumar, & Leslie, 2017), is a negative prognostic indicator in acute leukemias, including childhood B-ALL (Ruvolo, 2017; Tovar, Zeron, Romero, Sanchez, & Romero, 2016). These findings have provided a rationale for exploring the efficacy in the clinic of small molecules targeting this pathway in association with chemotherapy in B-ALL patients (Daver et al., 2015).

In this article, following a brief overview of what is known about PI3K/Akt/mTOR signaling in general and in B-ALL in particular, we will summarize the recent advances on its relevance as a potential therapeutic target in the various subtypes of B-ALL, with a particular emphasis on the combinatorial use of PI3K/Akt/mTOR inhibitors as novel therapeutic strategies.

## 2 | PI3K/Akt/mTOR SIGNALING

The PI3K family comprises lipid kinases that phosphorylate the 3′-OH of the inositol ring. PI3K isoforms are grouped into three classes, displaying distinct substrate specificity, and lipid products (Cantley, 2002). For the scopes of this review, we will mainly focus on class I PI3Ks as they are the best understood and the most widely implicated in human malignancies (Engelman, Luo, & Cantley, 2006; Millis, Ikeda, Reddy, Gatalica, & Kurzrock, 2016). Class I PI3Ks phosphorylate phosphatidylinositol-4,5-bisphosphate (PI(2)P) to yield phosphatidylinositol-3,4,5-trisphosphate (PI(3)P). Class I PI3Ks are divided into two subgroups: A and B. Class IA PI3Ks contain one of three catalytic subunits (p110α, p110β, p110δ) that form heterodimers with one of the five adaptor/regulatory isoforms (p85α, p85β, p55α, p55γ, p50α). Class IA PI3Ks are activated downstream of either tyrosine kinase receptors (TKRs) or G protein-coupled receptors (GPCRs). The single class IB PI3K comprises the p110γ catalytic subunit which binds one of two related regulatory subunits, either p101 or p87. Class IB PI3Ks mainly act downstream of GPCRs; however they can be stimulated by TKRs (Franke, 2008; Vanhaesebroeck, Guillermet-Guibert, Graupera, & Bilanges, 2010). Upon activation by growth factors, hormones, and...
cytokines, class I PI3Ks initiate a cascade of events that promote cancer cell proliferation, survival, migration, drug-resistance, and glycolytic metabolism.

Class II PI3Ks can be activated by TKRs, cytokine receptors and integrins, however their specific functions in cancer are still not well understood (Falasca et al., 2017).

Class III PI3K exclusively only a heterodimeric enzyme consisting of a catalytic (Vps34) and an adapter (p150) subunit (Yu, Long, & Shen, 2015). Vps34 is implicated in the regulation of autophagy in response to cellular stresses.

Akt is a serine/threonine kinase belonging to the AGC protein kinase family. Akt is a critical effector of PI3K in carcinogenesis, as documented by the fact that about 400 proteins contain the consensus sequence for Akt phosphorylation. The heterogeneity of proteins potentially phosphorylated by Akt supports the key role in cell cycle progression, survival, mRNA translation, glycolysis, and angiogenesis (Manning & Toker, 2017; Martini, De Santis, Braccini, Gulluni, & Hirsch, 2014).

The recruitment of inactive Akt from the cytosol to the plasma membrane requires that the pleckstrin homology (PH) domain of Akt binds to PIP3 synthesized by PI3K. Akt is then phosphorylated at Thr 308 by phosphatidylinositol-dependent kinase 1 (PDK1) and at Ser 473 by mTOR complex 2 (mTORC2), resulting in full activation of Akt activity (Georgescu, 2010).

mTOR is a serine/threonine kinase which belongs to the PI3K-related kinase (PIKK) family (Memmott & Dennis, 2009; Shen et al., 2018). mTOR is the catalytic subunit of two distinct multiprotein complexes, referred to as mTOR complex 1 (mTORC1) and mTORC2, which display different sensitivity to rapamycin and differ in their protein composition.

mTORC1 is characterized by the interactions between mTOR and the regulatory associated protein of mTOR (Raptor) which regulates mTOR activity and functions as a scaffold for recruiting mTORC1 substrates. This complex also comprises DEP-domain-containing mTOR interacting protein (Deptor), prolin-rich Akt substrate 40-kDa (PRAS40), mammalian lethal with Sec13 protein 8 (mLST8), and Tti1/Tel2.

mTORC1 is sensitive to rapamycin and its analogs (rapalogs) that are allosteric mTORC1 inhibitors and do not target the mTOR catalytic site (Alayev & Holz, 2013; Kim et al., 2002; Oshiro et al., 2004). mTORC1 controls translation in response to growth factors/nutrients through the phosphorylation of p70S6 kinase (p70S6K) and 4E-BP1 (Browne & Proud, 2004; Ma & Blenis, 2009; Shahbazian et al., 2010). Importantly, mTORC1 represses autophagy, a lysosome-dependent degradation pathway which allows cells to recycle damaged or superfluous cytoplasmic content (Chen et al., 2010; Fu et al., 2017, 2018). As a consequence, cells produce metabolic precursors for macromolecular biosynthesis or ATP generation. In cancer cells, autophagy fulfills a dual role, because it can display both tumor-suppressing and tumor-promoting functions. Indeed, the autophagic machinery prevents necrosis and inflammation, that can lead to genetic instability and tumorigenesis (Galluzzi, Bravo-San Pedro, Levine, Green, & Kroemer, 2017; Sugiyama et al., 2017; Wang, Zhang, Luo, Ning, & Fang, 2018). However, autophagy may be important for cancer progression, by providing energy through its recycling mechanism during unfavorable metabolic circumstances, that are very common encountered by tumor cells (Janku, McConkey, Hong, & Kurzrock, 2011; Won et al., 2018).

mTORC2 comprises the rapamycin-insensitive companion of mTOR (Rictor), Deptor, protein observed with rictor-1 (Protor-1), mLST8, mammalian stress-activated protein kinase interacting protein (mSIN1), and Tti1/Tel2. mTORC2 is generally described as being insensitive to rapamycin/rapalogs. However, long-term (>24 hr) treatment of cancer cells with rapamycin/rapalogs could result in mTORC2 activity inhibition (Dunlop & Tee, 2009; Rosner & Hengstschläger, 2008).

mTORC1 activity is quite well understood and is mainly regulated through PI3K/Akt signaling (Ilagan & Manning, 2016), although MEK/ERK could be also involved (Zeng et al., 2018).

In contrast, the mechanisms that control mTORC2 have only begun to be revealed (Sparks & Guertin, 2010), however mTORC2 activation by growth factors requires PI3K, as pharmacological inhibition of PI3K decreased mTORC2 activity in vitro (Huang, Dibble, Matsuzaki, & Manning, 2008; Liu et al., 2015).

PI3K, Akt, and mTORC1/2 are linked to each other via regulatory feedback loops that restrain their simultaneous hyperactivation (Dunlop & Tee, 2009). A negative regulation of Akt activity by mTORC1 is dependent on p70S6K-mediated phosphorylation of insulin receptor substrate (IRS) –1 and –2 adaptor proteins, downstream of the insulin receptor (IR) or insulin-like growth factor-1 receptor (IGF-1R) (Bhaskar and Hay, 2007; Lang et al., 2010; Shah, Wang, & Hunter, 2004). IRS-1 and IRS-2 are normally required to activate class IA PI3Ks after stimulation of IR/IGF-1R tyrosine kinase activity. When mTORC1 is active, p70S6K phosphorylates IRS-1/-2 on Ser residues, targeting them for proteasomal degradation (Sriburi, Jackowski, Mori, & Brewer, 2004; Xu et al., 2008). Therefore, mTORC1 signaling inhibition by rapamycin/rapalogs blocks this negative feedback loop and hyperactivates Akt through PI3K and PDK1 (Alayev et al., 2017; Bertacchini et al., 2014).

PI3K/Akt/mTOR network function is mainly controlled by inositol lipid phosphatases (Rodgers, Ferguson, Mitchell, & Ooms, 2017). In particular, phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a major negative regulator of the newtwork as it removes the 3′-phosphate from PIP3, thereby antagonizing PI3K signaling (Xie, Naizabekov, Chen, & Tokay, 2016).

### 3 | THE PI3K/Akt/mTOR PATHWAY IN B-ALL

Activation of PI3K/Akt/mTOR signaling is a common event in B-ALL cell lines and patient samples.

In Ph⁺ leukemic cells, PI3K activation is dependent on the presence of a multiprotein complex that, besides p110 and p85 PI3K, comprises Bcr-Abl, its substrate c-CBL (i.e., the cellular homolog of the v-Cbl oncoprotein [Brehme et al., 2009]), and the adaptor proteins,
CRKL and c-CRK (Gotoh, Miyazawa, Ohyashiki, & Toyama, 1994; Sattler et al., 1996; Skorski et al., 1995). The leukemogenic potential of activated PI3K is supported by the evidence that deletion of both Pik3r1 and Pik3r2 (which encode for class IA PI3K p85α and p85β, respectively [Vanhaesebroeck, Vogt, & Rommel, 2010]) markedly impaired leukemogenic transformation in a murine model of Ph⁺ B-ALL (Kharas et al., 2004, 2008). Other models of activation of PI3K in Ph⁺ B-ALL have been proposed, including Src family kinases, Ras, and IRS-1 (Kharas & Fruman, 2005).

Downregulation of PTEN gene expression has been observed in a Bcr-Abl-expressing murine pre-B-cell line, whereas PTEN overexpression delayed Ph⁺ B-ALL development in mice (Peng et al., 2010). Nevertheless, these results could not be confirmed using primary samples from adult patients with Ph⁺ B-ALL. However, in human Ph⁺ B-ALL PTEN, although expressed, is functionally inactivated (see further on). The mechanisms of PI3K/Akt/mTOR signaling activation in Ph⁺ B-ALL are highlighted in Figure 1a.

Regarding Ph⁻ B-ALL, evidence suggests that upregulation of the PI3K/Akt/mTOR network could be dependent on constitutively active pre-B-cell receptor (pre-BCR) signaling that characterizes approximately 13% of Ph⁻ B-ALL cases, whereas most Ph⁻ B-ALL cases lack expression of functional pre-BCR (Eswaran et al., 2015; Geng et al., 2015). However, it is unclear how active pre-BCR could positively impact on PI3K/Akt/mTOR activity. Given that pre-BCR⁺ B-ALL patients exhibit significantly higher expression and activity of Src family kinases, it might be that upregulation of the PI3K/Akt/mTOR cascade involves Syk, Fyn, or Lyn kinases (Geng et al., 2015; Kohrer et al., 2016). Moreover, expression and activity of PTEN were low in pre-BCR⁺ B-ALL cells (Geng et al., 2015).

Interleukin-7 (IL-7) and IL-7 receptor (IL-7R) are essential for normal lymphocyte development and homeostasis, whereas aberrant IL-7/IL7R-mediated signaling promotes leukemogenesis (Oliveira et al., 2017). Interestingly, in Ph⁺ B-ALL gain-of-function mutations in IL-7Ra were associated with the aberrant expression of cytokine-receptor-like factor 2 (CRLF2). Mutant IL-7Ra formed a functional receptor with CRLF2 for thymic stromal lymphopoietin (TSLP), which is closely related to IL-7 (Shochat et al., 2011, 2014). Although no evidence was provided that these mutations would

![Image](image.png)

**FIGURE 1** Mechanisms of PI3K/Akt/mTOR signaling activation in B-ALL subtypes. (a) Ph⁺ B-ALL. In this subtype, the Bcr-Abl substrate, c-CBL, forms a multimeric complex consisting of Bcr-Abl itself, both p85 and p110 PI3K, as well as the adaptor proteins, CRKL and c-CRK. The complex increases PIP3 production from PIP2 through PI3K activity (Sattler et al., 1996). In a murine model of Ph⁺ B-ALL, it has also been reported that Bcr-Abl downregulated PTEN gene expression, which resulted in increased levels of PIP3 (Peng et al., 2010). However, this mechanism of activation has not been confirmed using primary human Ph⁺ B-ALL samples, where PTEN protein was upregulated but functionally inhibited (Gomes et al., 2014). (b) Ph⁻ B-ALL. In this subset of B-ALL, the aberrant upregulation of the PI3K/Akt/mTOR network could be due to TSLP/mutated IL-7R/CRLF2 signaling (Shochat et al., 2011, 2014), ETV6/RUNX1 fusion gene (Fuka et al., 2012), disregulated pre-BCR signaling through Fyn, Lyn, or Syk kinases (Geng et al., 2015; Kohrer et al., 2016), or increased CK2 activity ensuing in PTEN phosphorylation and inhibition of its lipid phosphatase activity on PIP3 (Gomes et al., 2014). (c) Ph-like B-ALL. Here, multiple genetic aberrations of LNK/SH2B3, CRLF2, JAK 1/2, IL-7Ra, Abl 1/2, PDGFRB, CSF1R, and EPOR have the potential for activating PI3K/Akt/mTOR signals (Tasian et al., 2017).
result in PI3K/Akt/mTOR signaling activation, it is worth highlighting here that findings from other groups documented how TSLP promoted B-ALL cell survival and proliferation through the upregulation of the PI3K/Akt/mTOR network (Brown et al., 2007; Tasian et al., 2012). It is therefore plausible that IL-7Ra mutations could be one of the determinants of aberrant activation of the PI3K/Akt/mTOR cascade in Ph− B-ALL.

The ETV6/RUNX1 fusion gene is present in approximately 25% of childhood B-ALL patients and represents the molecular equivalent of the t(12;21) translocation (Romana et al., 1995). Down-regulation of the endogenous ETV6/RUNX1 fusion protein with shRNA in B-ALL cell lines resulted in suppression of cytokine-mediated PI3K/Akt/mTOR signaling. However, no mechanistic explanation of how ETV6/RUNX1 protein could impact on PI3K/Akt/mTOR cascade was provided by the authors (Fuka et al., 2012).

As to PTEN, it has been demonstrated that adult Ph− and Ph+ both B-ALL primary samples decreased PTEN activity and constitutive hyperactivation of PI3K/Akt/mTOR pathway despite high PTEN protein levels (Gomes et al., 2014). PTEN functional inactivation was dependent on CK2 that targets several Ser/Thr residues located at the C-terminal tail of PTEN (Buontempo et al., 2018). Indeed, treatment of B-ALL primary fusion protein and cell lines with the CK2 inhibitor CX-4945 resulted in decreased PTEN phosphorylation, downregulation of PTEN protein expression, increased PTEN lipid phosphatase activity, and Akt dephosphorylation. These results indicated that CK2 activity could be responsible for PTEN post-translational inactivation and upregulation of the PI3K/Akt/mTOR network in B-ALL cells (Gomes et al., 2014).

Overall, these findings have documented that multiple mechanisms could be involved in activation of PI3K/Akt/mTOR signaling in Ph− B-ALL (Figure 1b).

As to Ph-like B-ALL, aberrant PI3K/Akt/mTOR signaling has been reported in this disease subset which frequently displays a wide array of genetic anomalies which result in upregulation of signaling pathways downstream of LNK/SH2B3, CLRF2, JAK 1/2, IL-7Rα, Abl 1/2, platelet-derived growth factor receptor-β (PDGFRB), colony-stimulating factor-1 receptor (CSF1R), and erythropoietin receptor (EPOR) (Cheng et al., 2016; Reshmi et al., 2017). All of these aberrations have the potential for driving PI3K/Akt/mTOR signaling (Maude et al., 2012; Tasian et al., 2017; Vo et al., 2017) (Figure 1c).

4 | THERAPEUTIC TARGETING OF PI3K/Akt/mTOR IN PRECLINICAL MODELS OF B-ALL

Many small molecules targeting different nodes of the PI3K/Akt/mTOR signaling cascades have been or are in clinical development, however it is still unclear which of the different drugs could provide the greatest therapeutic index (Rodon & Tabernero, 2017). We will now review the different classes of inhibitors which have been tested in preclinical models of B-ALL subtypes (Table 1 and Figure 2).

5 | PI3K INHIBITORS

PI3K inhibitors comprise pan p110 PI3K inhibitors and isoform-specific inhibitors. The first report showing that a pan p110 PI3K inhibitor (LY294002) could induce either cell cycle arrest or apoptosis in a Ph− B-ALL cell line, RS4;11, dates back to 2004 when it was observed that Akt activation was required for the protective effects exerted by human stromal cells on B-ALL cells exposed to chemotherapeutic drugs such as Ara-C and etoposide (Wang, Fortney, & Gibson, 2004). These results were confirmed by another group which demonstrated, using BLIN-2 B-ALL cells, that either LY294002 or rapamycin, were proapoptotic only when combined with U0126, a MEK inhibitor. In contrast, when these drugs were combined with a JAK2 inhibitor (AG490) they were cytostatic, as they blocked cell proliferation, even when leukemic cells were co-cultured with stromal cells (Bertrand, Spengemen, Shelton, & McCubrey, 2005). However, LY294002 has not been clinically developed, mainly due to solubility problems.

The clinical grade pan p110 PI3K inhibitor BKM-120 (Buparlisib) has been tested in long-term cultures of primary Ph− and Ph+ B-ALL cells (Badura et al., 2013). BKM-120 inhibited both cell cycle arrest and apoptosis. Nevertheless, its antiproliferative effects were more evident in Ph− cells when compared to Ph+ cells, whereas apoptosis induction was stronger in Ph+ samples. More recently, the proapoptotic activity of BKM-120 in Ph− NALM-6 B-ALL cells was linked to upregulation of p53-regulated genes (Bashash et al., 2016).

Selective inhibitors, targeting one or two of the p110 PI3K catalytic subunits, have been synthesized with the hope that they would result in lower systemic toxicities than pan p110PI3K inhibitors (Hirsch & Chiarle, 2012). In consideration of the high levels of expression of p110δ PI3K in healthy lymphocytes (Chiu et al., 2017), inhibitors selectivity targeting this p110 PI3K isoform have been tested in preclinical models of Ph+ B-ALL. CAL-101 (Idelalisib) is a p110δ PI3K inhibitor whose use has been approved by the U.S. Food and Drug Administration (FDA) in combination with rituximab, an anti-CD20 antibody, as a second-line treatment for relapsed chronic lymphocytic leukemia (CLL) patients (Jones et al., 2017).

CAL-101 exerted cytotoxic effects against Nalm-6 B-ALL cells mediated by G1 blockage (presumably as a result of upregulated p21) and induction of caspase-dependent apoptosis, likely through reactive oxygen species-dependent upregulation of FOXO3a and subsequent induction of the proapoptotic target genes of p53 (Safaroghi-Azar, Bashash, Sadreazami, Momeny, & Ghaffari, 2017). Furthermore, CAL-101 increased the cytotoxicity of either doxorubicin (Safaroghi-Azar et al., 2017) or vincristine (Adam et al., 2017). Importantly, CAL-101 was able to decrease Akt phosphorylation at Ser 473 which was increased when B-ALL cell lines were co-cultured with OP-9 stromal cells (Adam et al., 2017). Furthermore, CAL-101 inhibited B-ALL cell migration to CXCL-12 (SDF-1α) in vitro and blocked homing of B-ALL cells to the bone marrow in vivo. It was thus concluded that inhibition of homing of circulating B-ALL cells to the bone marrow or even mobilization of leukemic cells from their protective niches in the bone marrow might interfere with cell adhesion-mediated drug-resistance...
Overall, these findings support the rationale for clinical testing of p110δ PI3K inhibitors in B-ALL and provide useful insights needed to optimize the therapeutic strategy.

Glucocorticoids (GCs) are widely used in chemotherapeutic protocols for B-ALL, due to their strong proapoptotic activity to leukemic cells. It was recently disclosed that dexamethasone (DEX) partially suppresses the PI3K/Akt signaling cascade in B-ALL cells. This results in nuclear translocation of the Akt substrate FOXO3a, which induces the expression of downstream targets, including p27Kip1 and Bim (Consolaro et al., 2015). We have recently studied the therapeutic potential of a panel of pan and isoform-selective p110 PI3K inhibitors, used alone or combined with DEX, in B-ALL leukemia cell lines and primary pediatric samples. We found that ZSTK-474, a pan p110 PI3K inhibitor, displayed the most powerful cytotoxic effects in B-ALL cells. However, both ZSTK-474 and the dual γ/δ p110 PI3K inhibitor IPI-145 (Duvelisib), sensitized B-ALL cells to DEX by restoring nuclear TABLE 1

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Target</th>
<th>Synergism with</th>
<th>B-ALL subtype</th>
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<td>Class IA PI3Ks</td>
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<td>PF05212384</td>
<td>PI3K/mTOR</td>
<td>Ruxolitinib, Dasatinib</td>
<td>Ph-like B-ALL</td>
<td>Tasian et al. (2017)</td>
</tr>
<tr>
<td>PP242</td>
<td>mTORC1/mTORC2</td>
<td>Dasatinib</td>
<td>Ph− B-ALL</td>
<td>Janes et al. (2010)</td>
</tr>
<tr>
<td>TAK-228</td>
<td>mTORC1/mTORC2</td>
<td>Dasatinib</td>
<td>Ph⁺, Ph− and Ph-like B-ALL</td>
<td>Janes et al. (2013); Vo et al. (2017); Yun et al. (2016)</td>
</tr>
<tr>
<td>AZD2014</td>
<td>mTORC1/mTORC2</td>
<td>-</td>
<td>Ph-like B-ALL</td>
<td>Tasian et al. (2017)</td>
</tr>
<tr>
<td>OSI-027</td>
<td>mTORC1/mTORC2</td>
<td>-</td>
<td>Ph⁺ and Ph− B-ALL</td>
<td>Yun et al. (2016)</td>
</tr>
<tr>
<td>Torin-2</td>
<td>mTORC1/mTORC2</td>
<td>-</td>
<td>Ph− B-ALL</td>
<td>Simioni et al. (2014)</td>
</tr>
</tbody>
</table>
translocation of the GC receptor and counteracted stromal cell-induced DEX-resistance (Evangelisti et al., 2018). Moreover, we performed gene expression analysis which demonstrated that the combination consisting of ZSTK-474 and DEX strengthened the DEX-induced up- or downregulation of several genes involved in apoptosis, however the combination also rescued the effects of genes that might be involved in GC-resistance. Another group has recently demonstrated that CAL-101 synergizes with DEX in Ph⁺ B-ALL cell lines and primary samples from DEX-resistant patients (Kruth et al., 2017).

It has been proposed that PI3K/Akt/mTOR signaling is involved in the resistance of Ph⁺ B-ALL cells to the TKI, imatinib (Guo et al., 2017; Xing et al., 2012; Wang et al., 2014). For this reason, our group has recently investigated the therapeutic potential of combining PI3K inhibitors with TKIs in the Ph⁺ B-ALL cell line SUP-B15. We used the pan p110 PI3K inhibitor ZSTK-474, the p110α PI3K inhibitor BYL719 and the dual γ/δ p110 PI3K inhibitor IPI-145. As to TKIs, we employed imatinib, the second generation Bcr-Abl inhibitor nilotinib and the third generation orally bioavailable TKI, GZD824 (Ultimo et al., 2017). Of note, GZD824 is effective against a wide range of Bcr-Abl mutants, including T315I (Ren et al., 2013). We observed synergistic cytotoxic effects with the various combinations which were due to apoptosis induction. However, the combined treatments also induced autophagy, which might have a protective effect (Ultimo et al., 2017). In this connection, it is important to emphasize how an ATP-competitive dual inhibitor of PI3K p110δ/Vps34, PI3KD/V-IN-01, exhibited better antiproliferative activity against cell lines representative of B-cell malignancies than known selective PI3K p110δ and Vps34 inhibitors (Liu et al., 2016). PI3KD/V-IN-01 has not been tested in B-ALL preclinical settings, however, its use should be considered in light of our own findings (Ultimo et al., 2017).

Overall, all of the above highlighted results support the rationale for clinical testing of either pan p110 or isoform-selective PI3K inhibitors in B-ALL patients, especially in combination with traditional chemotherapeutic drugs or TKIs, and provide useful insights needed to optimize the therapeutic strategy, including the protective role played by autophagy when leukemic cells are treated with PI3K inhibitors.

5.1 | Akt inhibitors

Historically, Akt has been a difficult target to inhibit with ATP-competitive small molecules. Indeed, the ATP-binding pocket of Akt shares many homologies with other members of the AGC kinase family (Pearce, Komander, & Alessi, 2010). GSK690693 is an ATP-competitive inhibitor which targets Akt (Rhodes et al., 2008). GSK690693 has

**FIGURE 2** Small molecule inhibitors used for targeting the key nodes of the PI3K/Akt/mTOR cascade in preclinical models of B-ALL. For the details, see the text.
been tested in Ph+ and Ph− B-ALL cell lines where it induced a cell proliferation block and apoptosis accompanied by a dephosphorylation of Akt downstream targets, including GSK3β, PRAS40, and p70S6K (Levy, Kahana, & Kumar, 2009). Importantly, both stimulated peripheral blood CD4+ T-cells from healthy donors and mouse thymocytes were less sensitive to GSK690693 than leukemic cells. Nevertheless, dose-limiting toxicities that are likely on target, associated with hyperglycemia, led to the terminal of the clinical development of this drug (Manning & Toker, 2017). MK2206 is a specific, potent, and orally bioavailable allosteric inhibitor that targets both the catalytic and the PH domains of Akt (Hirai et al., 2010). We have shown that MK2206 strongly synergized with the mTORC1 inhibitor, RAD001, in both Ph− B-ALL cell lines and patient samples (Neri et al., 2014). Our findings could have important implications, as MK2206 has not shown significant efficacy in the clinic as single agent therapy in patients with acute myelogenous leukemia (Konopleva et al., 2014). However, its effects could be conceivably enhanced by other drugs used in combination therapy.

5.2 | Allosteric mTOR inhibitors

Allosteric mTOR inhibitors (rapamycin, CCI-779, RAD001) have been widely used in preclinical models of B-ALL. For example, rapamycin negatively affected in vitro the growth of Eμ-ret transgenic mouse-derived cell lines as well as that of Ph+ human B-ALL cell lines (NALM-6, NALM-16). Moreover, rapamycin induced apoptosis in the murine pro-B ALL cell line T309 (Brown et al., 2003). Interestingly, the inhibitory effects of rapamycin could be reversed by IL-7, which suggests an important role of this cytokine in the control of mTOR activity in B-ALL cells. Rapamycin was also effective in vivo, as it extended survival in Eμ-ret transgenic mice with advanced B-ALL compared with control littermates (Brown et al., 2003).

In vitro, CCI-779 (Temsirolimus) dramatically decreased survival and increased apoptosis of lymphoblasts from Ph- B-ALL adult patients co-cultured with bone marrow stromal cells. The drug was also effective in vivo in a NOD/SCID xenograft model (Teachey et al., 2006). Recently, CCI-779 has been shown to synergize with the Bcl-2 inhibitor, ABT-737, in ABT-737-resistant pediatric and adult Ph− B-ALL cells, by downregulating mTORC1 activity and Mcl-1 (Iacovelli et al., 2015).

Also RAD001 has proven its efficacy, especially in models of Ph− pediatric B-ALL. Importantly, RAD001 synergized with conventional chemotherapy (e.g., vincristine) or novel agents (e.g., bortezomib) both in vitro and in vivo, with increased caspase-dependent, but p53-independent, cell killing (Saunders, Cisterne, Weiss, Brandstock, & Bendall, 2011; Saunders et al., 2013). However, it has also been shown that RAD001 induces other types of cell death, including autophagy (Crazzolara, Brandstock, & Bendall, 2009) and caspase-independent apoptosis (Baraz et al., 2014).

It should be considered that interactions between leukemic cells and the bone marrow microenvironment promote B-ALL cell survival and confer resistance to chemotherapy (Chiarini et al., 2016). Hypoxia is an integral component of bone marrow microenvironment (Petit et al., 2016). Hypoxia-inducible factor-1α (HIF-1α) plays key roles in response to hypoxia by regulating leukemic cell growth and metabolic adaptation to hypoxia, as well as drug-resistance (Deynoux, Sunter, Herault, & Mazurier, 2016). Remarkably, mTOR blockade with RAD001 reduced HIF-1α expression, diminished glucose uptake and glycolytic rate, and partially restored the chemosensitivity of B-ALL cells co-cultured with bone marrow stromal cells under hypoxic conditions (Frolova et al., 2012).

In this connection it is important to emphasize that cancer cells, including acute leukemia cells, show the propensity to metabolize glucose to lactate for ATP production even under aerobic conditions, the so-called “Warburg effect” (Kalyanaraman, 2017). Therefore, drugs targeting glycolysis are now being studied for developing innovative anticancer therapies (Granja, Pinheiro, Reis, Martinho, & Baltazar, 2015). mTORC1 senses metabolic stresses, such as lowered glucose metabolite levels, and ATP concentrations, and compensates for these stresses, allowing cells to become resistant to glycolysis inhibition (Jellusova & Rickert, 2016). Interestingly, rapamycin potentiated the effects of the novel, third-generation glycolysis inhibitor, 3-bromo-2-oxopropionate-1-propyl ester (3-BrOP), a cell permeable ester of 3-bromopyruvate (3-BrPA) (Lis et al., 2016). NALM-6 B-ALL cells treated with 3-BrOP and rapamycin displayed a decrease in viability compared to either agent alone (Akers et al., 2011). These findings suggest that when ATP is depleted by glycolysis inhibition, simultaneous targeting of mTORC1 may further limit nutrient uptake, ensuing in stronger cytotoxic effects.

Rapamycin has shown efficacy in preclinical models of xenografted pediatric Ph-like B-ALL with and without CRLF2 and JAK genetic lesions. Indeed, the drug controlled leukemia burden in all eight B-ALL samples analyzed, while a prolonged survival was observed in two representative xenografts treated with rapamycin when compared with vehicle (Maude et al., 2012).

As to Ph+ B-ALL, it has been shown that rapamycin was cytotoxic not only for the Bcr-Abl+ leukemic lymphoid cell line, SU-Ph2, but also for the imatinib-resistant cell line, SU/SR, that has acquired the T315I mutation (Hirase, Maeda, Takai, & Kanamaru, 2009). Moreover, rapamycin potentiated the effects of imatinib in SUP-B15 Ph− B-ALL cells and primary samples from patients when combined with daunorubicin (Yang et al., 2012). Remarkably, it was reported that daunorubicin treatment up-regulated mTORC1 signaling, whereas rapamycin effectively counteracted such a deleterious side effect, which might dampen daunorubicin ability to kill leukemic cells. The same group subsequently reported that imatinib enhanced mTORC1 signaling, therefore its association with rapamycin increased imatinib cytotoxicity in SUP-B15 cells (Yang et al., 2014).

Importantly, it has also been shown that RAD001 overcame resistance to imatinib by targeting in vitro and in vivo a mostly quiescent Ph+ B-ALL cell subset (CD34+/CD38−) (Kuwatsuka et al., 2011). It is well known that this cell subset is enriched in leukemic stem cells (LSCs) that are intrinsically resistant to TKIs, probably, although not exclusively, due to their quiescence (Graham et al., 2002).

The RAD001/imatinib combined treatment induced in vitro apoptosis of CD34+/CD38− cells more efficiently than RAD001 alone.
(Kuwatsuka et al., 2011). These findings imply that co-treatment with RAD001 can overcome resistance to imatinib in Ph⁺ B-ALL LSCs and may pave the way for more effective treatments aimed to lower the percentage of patients who relapse after TKI treatment.

Together, all these preclinical observations suggest that allosteric mTOR inhibitors, especially when combined with conventional chemotherapy or novel targeted agents, have the potential to improve clinical responses in patients with different types of B-ALL, including high risk Ph⁺ and Ph-like cases.

5.3 | Dual PI3K/mTOR inhibitors

Since both PI3K and mTOR belong to the PIKK superfamily of kinases, they share high sequence homology in their catalytic domains. Dual PI3K/mTOR inhibitors were originally developed as PI3K inhibitors, but were subsequently shown to also effectively inhibit both mTORC1 and mTORC2 (Chiarini, Evangelisti, McCubrey, & Martelli, 2015). The use of this class of drugs should blunt mTORC2-dependent activation of Akt effected through PI3K (Janes et al., 2010).

PI-103, BEZ235, BTG226, and PF05212384 (Gedatolisib) are dual PI3K/mTOR inhibitors that have been employed in preclinical models of B-ALL.

PI-103 was more efficacious than rapamycin at suppressing proliferation of murine and human Ph⁺ B-ALL leukemia cells treated with the TKI inhibitor, imatinib (Kharas et al., 2008). BEZ235 displayed cytotoxic effects in both Ph⁻ (Schult et al., 2012; Wong, Welschinger, Hewson, Bradstock, & Bendall, 2014) and Ph⁺ B-ALL cells (Ding et al., 2013), while BTG226 has been tested only in Ph⁺ models, where it was more powerful than BEZ235 (Wong et al., 2014). Both drugs, however, inhibited the proliferation of Ph⁺ B-ALL cell lines with a three log greater potency than RAD001 (Wong et al., 2014). BEZ235 synergized with traditional chemotherapeutic drugs, including cytarabine, doxorubicin, and DEX in Ph⁻ B-ALL cells (Schult et al., 2012), while the inhibitor was synergistic with the second-generation TKI, nilotinib, in Ph⁺ SUP-B15 cells. Interestingly, nilotinib, when administered alone, upregulated PI3K/Akt/mTOR signaling (Ding et al., 2013). Moreover, BEZ235 has been shown to synergize with the Bcl-2 inhibitor, GX15-070 (Obatoclax), in Ph⁻ and Ph⁺ primary B-ALL cells and cell lines (Stefanzl et al., 2017).

While the clinical development of BEZ235 and BTG226 has been halted, that of PF05212384 is being continued (Rodon & Tabernero, 2017). Remarkably, PF05212384 displayed antitumor activity in childhood B-ALL patient-derived xenograft models harboring various Ph-like genomic alterations (Tasian et al., 2017). PF05212384 was particularly effective in CRLF2/JAK-mutant models with a mean 92.2% leukemia proliferation reduction versus vehicle controls and significantly prolonged mice survival. It displayed a lower efficacy in Abl/PDGFR-mutant models. Overall, PF05212384 was more potent than the p110α PI3K inhibitor BYL719, the p110δ PI3K inhibitor CAL-101, or the mTORC1/mTORC2 inhibitor AZD2014. Nevertheless, the efficacy of PF05212384 monotherapy was enhanced by combining the PI3K/mTOR inhibitor with either a JAK inhibitor (Ruxolitinib) in CRLF2/JAK-mutant models or the TKI dasatinib in Abl/PDGFR-mutant models (Tasian et al., 2017). These data suggested that Ph-like B-ALL requires PI3K/Akt/mTOR pathway activation for survival in vivo. Moreover, they indicated that clinical trials testing combinations of kinase inhibitors in Ph-like B-ALL patients are warranted.

6 | ATP-COMPETITIVE mTORC1/mTORC2 INHIBITORS

This class of drugs, that target both mTOR complexes but not PI3K, was designed to reduce toxicity due to the use of dual PI3K/mTOR inhibitors (Pal & Quinn, 2013). Moreover, these inhibitors can potentially prevent the feedback loop activation of Akt caused by rapamycin/rapalogs in B-ALL cells (Badura et al., 2013). In analogy with dual PI3K/mTOR inhibitors, the ATP-competitive mTORC1/mTORC2 inhibitor PP242, displayed more powerful cytotoxic effects as well as a more complete inhibition of mTORC1 outputs compared with rapamycin in preclinical models of murine and human Ph⁺ B-ALL cells (Janes et al., 2010).

TAK-228 (formerly known as INK128/MLN0128) is an orally active mTORC1/mTORC2 inhibitor currently in clinical development. TAK-228 suppressed proliferation of B-ALL cell lines in vitro and reduced colony formation by primary human leukemia cells from both adult and pediatric Ph⁺ B-ALL patients. TAK-228 also increased the efficacy of dasatinib in Ph⁺ primary samples (Janes et al., 2013). This inhibitor was also effective in various in vivo models of B-ALL, including a syngeneic mouse model of lymphoid Ph⁺ disease, and primary xenografts of either Ph⁺ or Ph⁻ human B-ALL samples. Remarkably, in all the in vivo models, TAK-228 was well tolerated and did not suppress endogenous bone marrow proliferation (Janes et al., 2013). Very recently, however, it has been shown that inhibiting mTORC1 with TAK-228 protected Ph⁺ and Ph-like B-ALL cells from killing by methotrexate and 6-mercaptopurine, two drugs used in maintenance chemotherapy. These cytoprotective effects of TAK-228 correlated with decreased cell-cycle progression and were also detected with cell-cycle inhibitors, such as palbociclib or aphenicolin (Vo et al., 2017). Using xenograft models, it was observed that mTOR inhibition by TAK-228 actually increased the number of leukemia cells that emerged after cessation of chemotherapy treatment.

Interestingly, the use of TAK-228 and shRNA-mediated down-regulation of either Raptor or Rictor allowed the identification of two previously unrecognized mTOR-dependent pathways critical for Ph⁺ and Ph⁻ B-ALL cell survival. Inhibition of mTORC1-mediated 4E-BP1 phosphorylation led to decreased expression of c-Myc and subsequent upregulation of the proapoptotic Bcl-2 family member PUMA, whereas inhibition of mTORC2 resulted in NF-κB-mediated expression of the Early Growth Response 1 (EGR1) gene, that encodes a transcription factor which binds and transactivates the proapoptotic BCL2L11 gene encoding Bim protein (Yun et al., 2016). Importantly, 1 or both pathways contributed to apoptosis of malignant B-ALL cells after treatment with the dual mTORC1/mTORC2 inhibitors, OSI-027 and TAK-228.
Our group has documented that the ATP-competitive mTORC1/mTORC2 inhibitor, Torin-2, was cytotoxic to a panel of Ph- B-ALL cell lines and indeed suppressed the rapalog-dependent feedback activation of Akt, whereas RAD001 required the addition of the Akt inhibitor MK-2206 to achieve the same effects (Simioni et al., 2014).

Overall, these findings support the rationale for clinical testing of ATP-competitive mTORC1/mTORC2 inhibitors in both Ph- and Ph+ B-ALL patients. However, they also highlight that inhibitors targeting mTOR should be used with caution in combination with chemotherapeutic drugs that rely on cell-cycle progression to kill leukemic cells.

7 | CONCLUSIONS AND FUTURE DIRECTIONS

The evidence reviewed here documents that PI3K/Akt/mTOR-generated signals play central roles in the control of B-ALL cell proliferation, survival, metabolism, and drug-resistance, making this signal transduction cascade an attractive target for antileukemic therapy. These findings have highlighted PI3K/Akt/mTOR inhibitors as molecules to be possibly tested in clinical trials aimed to treat B-ALL patients, although several fundamental outstanding issues still need to be addressed.

In particular, will inhibition of PI3K/Akt/mTOR signaling negatively affect leukemic cells without deleterious side effects on healthy cells? In other words, is there a therapeutic window when such a ubiquitous signal transduction cascade is to be targeted? While the discovery of PI3K/Akt/mTOR inhibitors yielded a flood of promising and exciting preclinical data, the initial wave of clinical trials based on these drugs has not resulted in widespread and durable patient responses. These trials have demonstrated that PI3K/Akt/mTOR pathway inhibitors frequently displayed significant on- and off-target toxicities that were dose-limiting, often leading to the use of subtherapeutic maximum tolerated doses, which resulted in insufficient pathway inhibition. Rapamycin/rapalogues were generally quite well tolerated, however they have shown a modest anticancer activity when used alone. This low efficacy is usually explained by the feedback activation of Akt, as we have discussed in this review.

As a consequence of these trials, only a handful of these inhibitors have been approved for clinical use as anticaner agents in the U.S. and Europe (Chiarini et al., 2015; Rodon & Tabernero, 2017). The depth and duration of target inhibition as well as safety profiles of these compounds may be improved through the use of intermittent dosing schedules, which could lead to a better drug exposure with more effective target inhibition and fewer off-target adverse effects, as seen in other types of cancer (Guichard et al., 2015; Tolcher et al., 2015; Alayev, A., & Holz, M. K. (2013). mTORC1/mTORC2 inhibition for biological control and cancer. Journal of Cellular Physiology, 228, 814–820).

Furthermore, preclinical data strongly suggest that identifying combinations, either with targeted agents or with chemotherapy, might be the key to unleashing the full potential of PI3K/Akt/mTOR inhibitors in B-ALL patients, as we have highlighted in this review. Early clinical data support this claim in other cancer types (Andre et al., 2014; Moscetti et al., 2016), although it will have to be conclusively demonstrated that improved clinical responses will not be accompanied by unacceptable toxicities (Baselga et al., 2017).

Another critical issue will be the identification of biomarkers that could accurately predict PI3K/Akt/mTOR pathway inhibitor efficacy in B-ALL patients. In this connection, techniques such as kinase activity profiling and computational analysis could give a deeper insight in active signal transduction pathways and point out critical signaling hubs as well as new potential druggable targets or resistant patients, as recently demonstrated also in B-ALL cells (Casado et al., 2013; van der Sligte et al., 2015).

We also need to better understand the effects of the pathway inhibitors on the immunosuppressive leukemic microenvironment. Indeed, recent evidence pointed out that B-ALL cells induced inhibition of T-cell Akt/mTORC1 signaling and glucose metabolism that drove T-cell function impairment, while an enforced Akt/mTORC1 signaling rescued T-cell metabolism and partially improved anti-leukemia immunity (Siska et al., 2016). Therefore, the use of PI3K/Akt/mTOR inhibitors could further blunt immunological responses against leukemic cells.

In conclusion, the field of PI3K/Akt/mTOR targeted therapies has progressed very rapidly over the past 15 years and, as our knowledge of the biology continuously increases, so too will our capacity to improve and finely tune these novel therapies to positively affect B-ALL patient outcome.

CONFLICTS OF INTEREST

None declared.

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