3

The PI3K/PKB Signaling Module in Normal and Malignant Hematopoiesis

Roel Polak[1] and Miranda Buitenhuis[1,2]
[1]*Department of Hematology, Erasmus MC, Rotterdam,*
[2]*Erasmus MC Stem Cell Institute for Regenerative Medicine, Erasmus MC, Rotterdam, the Netherlands*

1. Introduction

Hematopoiesis is a complex series of events resulting in the formation of mature blood cells. This process is regulated by cytokines at various levels, including self-renewal, proliferation, and differentiation. Upon binding of cytokines to their cognate receptors, the activity of intracellular signal transduction pathways is regulated, leading to modulation of gene expression. Although our appreciation of the transcriptional regulators of hematopoiesis has developed considerably, until recently, the roles of specific intracellular signal transduction pathways were largely unknown. An important mediator of cytokine signaling implicated in regulation of hematopoiesis is the Phosphatidylinositol-3-Kinase (PI3K) / Protein Kinase B (PKB/c-Akt) signaling module (Figure 1).

The PI3K family consists of three distinct subclasses of which, to date, only the class I isoforms have been implicated in regulation of hematopoiesis. Four distinct catalytic class I isoforms have been identified; p110α, p110β, p110δ and p110γ (reviewed by Vanhaesebroeck et al., 2001). These isoforms are predominantly activated by protein tyrosine kinases and form heterodimers with a group a regulatory adapter molecules, including p85α, p85β, p50α p55α, p55γ and p101γ (reviewed by Vanhaesebroeck et al., 2001). The most important substrate for these Class I PI3Ks is phosphatidylinositol 4,5 bisphosphate ($PI(4,5)P_2$) which can be phosphorylated at the D3 position of the inositol ring upon extracellular stimulation, resulting in the formation of phosphatidylinositol 3,4,5 trisphosphate ($PI(3,4,5)P_3$) (reviewed by Hawkins et al., 2006). $PI(3,4,5)P_3$ subsequently serves as an anchor for pleckstrin homology (PH) domain-containing proteins, such as Protein Kinase B (PKB/ c-akt) (Burgering & Coffer, 1995). Activation of PKB requires phosphorylation on both Thr^{308}, in the activation loop, by phosphoinositide-dependent kinase 1 (PDK1) and Ser^{473}, within the carboxyl-terminal hydrophobic motif, by the MTORC2 complex that consists of multiple proteins, including Mammalian Target of Rapamycin (mTOR) and Rictor (Sarbassov et al., 2005).

PKB itself subsequently regulates the activity of multiple downstream effectors, including the serine/threonine kinase Glycogen Synthase Kinase-3 (GSK-3) (Cross et al., 1995), members of the FoxO subfamily of forkhead transcription factors FoxO1, FoxO3, and FoxO4 (Brunet et al., 1999; Kops et al., 1999) and the serine/threonine kinase mammalian target of rapamycin (mTOR) as part of the MTORC1 complex, which also includes the regulatory

associated protein of mTOR (Raptor). In contrast to GSK-3 and the FoxO transcription factors that are inhibitory phosphorylated by PKB, activation of mTOR is positively regulated (Nave et al., 1999; Inoki et al., 2002). It has been demonstrated that PKB can inhibit the GTPase activating protein Tuberous sclerosis protein 2 (TSC2)/TSC1 complex, resulting in accumulation of GTP-bound Rheb and subsequent activation of mTOR (Inoki et al., 2002).

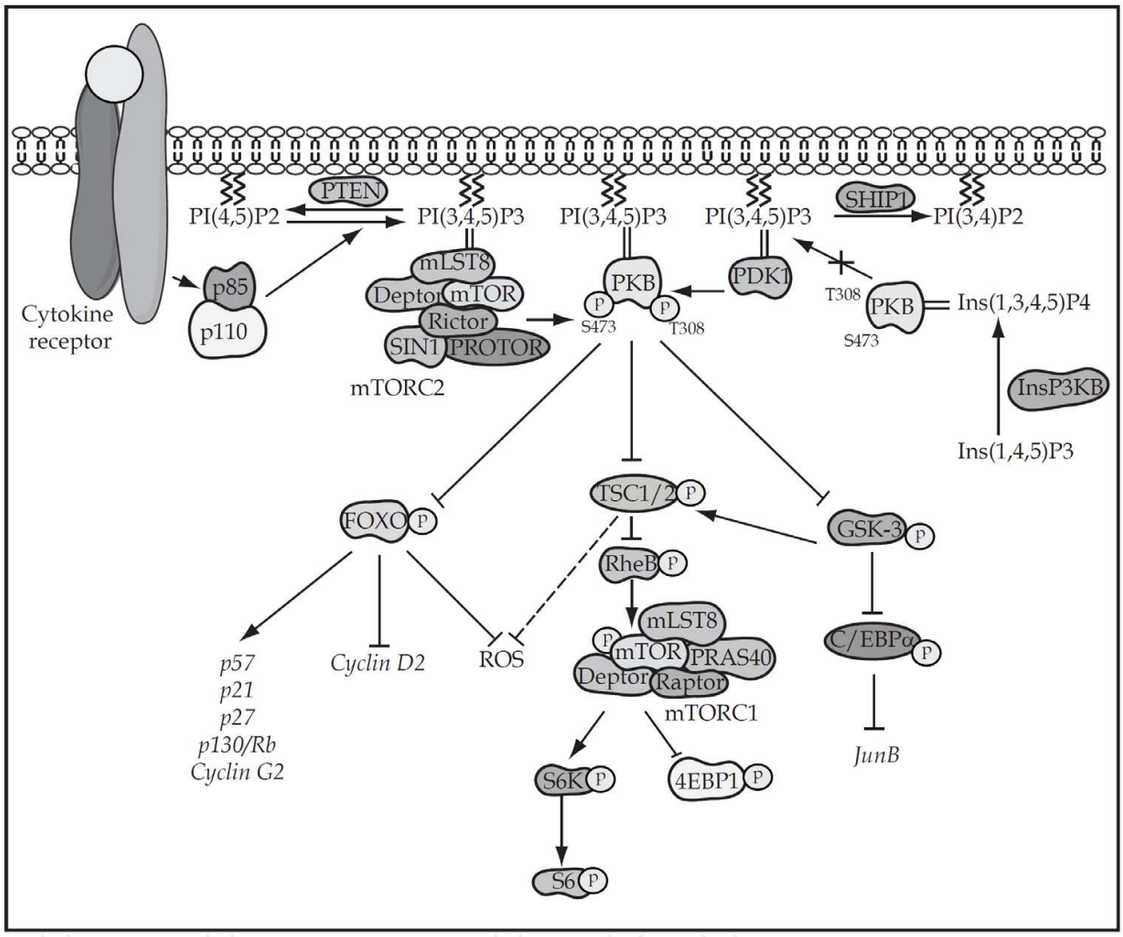


Fig. 1. *Schematic representation of the PI3K/PKB signaling module.* Activation of PI3K by receptor stimulation results in the production of PtdIns(3,4,5)P_3 at the plasma membrane. PKB subsequently translocates to the plasma membrane where it is phopshorylated by PDK1 and the mTORC2 complex. Upon phosphorylation, PKB is released into the cytoplasm where it can both inhibitory phosphorylate multiple substrates, including FoxO transcription factors and GSK-3 and induce the activity of other substrates such as mTOR as part of the mTORC1 complex. Negative regulators of the PI3K/PKB signaling module include PTEN, SHIP1 and Ins(1,3,4,5)P4.

While cytokines and growth factors positively induce PI3K activity, its activity can also be inhibited by SH2-containing inositol-5'-phosphatase 1 (SHIP1) (Damen et al., 1996), a protein predominantly expressed in hematopoietic cells (Liu et al., 1998), that hydrolyzes PIP_3 to generate PI(3,4)P_2 (Damen et al., 1996). Similarly, Phosphate and Tensin Homologue (PTEN) (Maehama & Dixon, 1998), a ubiquitously expressed tumor suppressor protein, can

dephosphorylate PIP_3 resulting in the formation of $PI(4,5)P_2$ (Maehama & Dixon, 1998). Although both PTEN and SHIP1 act on the main product of PI3K activity, PIP_3, the products generated are distinct. $PI(3,4)P_2$ and $PI(4,5)P_2$ both act as discrete second messengers activating distinct downstream events (Dowler et al., 2000; Golub & Caroni, 2005) indicating that the activation of SHIP1 and PTEN not only inhibit PI3K activity, but also can re-route the signal transduction pathways activated by PI-lipid second messengers.

2. PI3K/PKB signaling and normal hematopoiesis

2.1 PI3K

The role of PI3K class I isoforms was initially examined utilizing knockout mice deficient for one or multiple regulatory or catalytic subunits. Combined deletion of p85α, p55α and p50α resulted in a complete block in B cell development (Fruman et al., 2000). Similarly, introduction of a mutated, catalytically inactive p110δ ($p110\delta^{D910A}$) in the normal p110δ locus also resulted in a block in early B cell development while T cell development was unaffected (Jou et al., 2002; Okkenhaug et al., 2002). These results indicate that PI3K activity is essential for normal B lymphocyte development. Pharmacological inhibition of PI3K activity in human umbilical cord blood derived CD34+ hematopoietic stem and progenitor cells revealed that inhibition of the activity of PI3K is sufficient to completely abrogate both proliferation and differentiation during *ex vivo* eosinophil and neutrophil development eventually leading to cell death (Buitenhuis et al., 2008). Conditional deletion of either PTEN or SHIP1 in adult HSCs resulting in activation of the PI3K pathway not only reduced the level of B-lymphocytes but also enhanced the level of myeloid cells (Helgason et al., 1998; Liu et al., 1999; Zhang et al., 2006). In addition, these mice developed a myeloproliferative disorder that progressed to leukemia (Helgason et al., 1998; Liu et al., 1999; Zhang et al., 2006). Furthermore, enhanced levels of megakaryocyte progenitors have been observed in SHIP1 deficient mice (Perez et al., 2008). In PTEN heterozygote (+/-) SHIP null (-/-) mice, a more severe myeloproliferative phenotype, displayed by reduced erythrocyte and platelet numbers and enhanced white blood cell counts including elevated levels of neutrophils and monocytes in the peripheral blood, could be observed (Moody et al., 2004). Interestingly, PI3K appears not only to be involved in lineage development, but is also required for stem cell maintenance. In PTEN and SHIP1 deficient mice, an initial expansion of HSCs could be observed which was followed by a depletion of long-term repopulating HSCs (Damen et al., 1996; Helgason et al., 2003). Recently, a shorter SHIP1 isoform (s-SHIP1), which is transcribed from an internal promoter in the SHIP1 gene, has also been implicated in positive regulation of lymphocyte development during hematopoiesis. (Nguyen et al., 2011). Its role in regulation of HSCs and long-term hematopoiesis remains to be investigated (Nguyen et al., 2011). A third negative regulator of the PI3K/PKB signaling module is Inositol 1,3,4,5-tetrakiphosphate (Ins(1,3,4,5)P4), which is generated from Inositol 1,4,5-triphosphate (Ins(1,4,5)P3) by Inositol triphosphate 3-kinase B (InsP3KB). It has been shown that Ins(1,3,4,5)P4 can bind to the PIP3-specific PH domains and competes for binding to those PH domains with PIP3 (Jia et al., 2007). In the bone marrow of mice deficient for InsP3KB, an acceleration of proliferation of the granulocyte macrophage progenitor has been observed resulting in higher levels of GMPs and mature neutrophils (Jia et al., 2008). In addition, although B lymphocytes could still be observed in InsP3KB deficient mice, mature CD4+ and CD8+ T lymphocytes were almost completely absent (Pouillon et al., 2003). Although InsP3KB is also involved in regulation of other pathways, the enhanced PKB phosphorylation in these mice (Jia et al., 2008) suggest that the observed phenotype is at

least partially due to activation of the PI3K/PKB signaling module. Taken together, these studies suggest that correct temporal regulation of PI3K activity is critical for both HSC maintenance and regulation of lineage development.

2.2 PKB

PKB, an important effector of PI3K signaling, has been demonstrated to play an important role in regulation of cell survival and proliferation in a variety of systems (reviewed by Manning & Cantley, 2007). Three highly homologous PKB isoforms have been described to be expressed in mammalian cells; PKBα, PKBβ, and PKBγ. Analysis of HSCs derived from PKBα/PKBβ double-knockout mice revealed that PKB plays an important role in maintenance of long-term repopulating HSCs. These PKBα/PKBβ double-deficient HSCs were found to persist in the G_0 phase of the cell cycle, suggesting that the long-term functional defects observed in these mice were caused by enhanced quiescence (Juntilla et al., 2010). In contrast, loss of only one of the isoforms only minimally affected HSCs (Juntilla et al., 2010). In addition, analysis of mice deficient for both PKBα and PKBβ revealed that the generation of marginal zone and B1 B cells and the survival of mature follicular B cells highly depend on the combined expression of PKBα and PKBβ. Again no significant differences could be observed in mice deficient for the single isoforms (Calamito et al., 2010). In addition, ectopic expression of constitutively active PKB in mouse HSCs conversely resulted in transient expansion and increased cycling of HSCs, followed by apoptosis and expansion of immature progenitors in BM and spleen, which was also associated with impaired engraftment (Kharas et al., 2010), again demonstrating the importance of PKB in HSC maintenance. Utilizing an *ex vivo* human granulocyte differentiation system and a mouse transplantation model, it has recently been demonstrated that PKB not only plays a role in expansion of hematopoietic progenitors, but also has an important function in regulation of cell fate decisions during hematopoietic lineage commitment (Buitenhuis et al., 2008). High PKB activity was found to promote neutrophil and monocyte development and to inhibit B lymphocyte development, while conversely reduction of PKB activity is required to induce optimal eosinophil differentiation (Buitenhuis et al., 2008). In addition, PKB plays an important role in regulation of proliferation and survival of dendritic cell (DC) progenitors, but not maturation (van de Laar et al., 2010). Transplantion of mouse bone marrow cells ectopically expressing constitutively active PKB was sufficient to induce a myeloproliferative disease in most mice, characterized by extramedullary hematopoiesis in liver and spleen. In the majority of those mice, lymphoblastic thymic T cell lymphoma could also be observed. In addition, an undifferentiated AML developed in those mice that did not develop a myeloproliferative disease (Kharas et al., 2010).

2.3 Downstream effectors of PKB

To understand the molecular mechanisms underlying PKB mediated regulation of hematopoiesis, the roles of its downstream effectors in hematopoiesis have been investigated. FoxO transcription factors are known to play an important role in regulation of proliferation and survival of various cell types (reviewed by Birkenkamp & Coffer, 2003). Although proliferation and differentiation of hematopoietic progenitors appears not to be affected in FoxO3 deficient mice, competitive repopulation experiments revealed that deletion of FoxO3 is sufficient to impair long-term reconstitution (Miyamoto et al., 2007). In addition, in aging mice, the frequency of HSCs was increased compared to wild type littermate controls

(Miyamoto et al., 2007) and neutrophilia developed upon myelosuppressive stress conditions (Miyamoto et al., 2007). In contrast to FoxO3 deficient mice in which neutrophilia only occurred after myelosuppression while aging, conditional deletion of FoxO1, 3, and 4 in the adult hematopoietic system, was sufficient to increase the levels of myeloid cells and decrease the number of peripheral blood lymphocytes under normal conditions. In time, these mice developed leukocytosis characterized by a relative neutrophilia and lymphopenia (Tothova et al., 2007). In addition, an initial expansion of HSCs has been observed in these mice which correlated with an HSC-specific up-regulation of Cyclin D2 and down-regulation of Cyclin G2, p130/Rb, p27, and p21 (Tothova et al., 2007). Furthermore, a defective long-term repopulating capacity of bone marrow cells was observed, which could be explained by the reduction in HSC numbers that followed the initial expansion (Tothova et al., 2007). Although deletion of FoxO3 alone was not sufficient to improve myeloid development, ectopic expression of a constitutively active, non-phosphorylatable, FoxO3 mutant in mouse hematopoietic progenitors did result in a decrease in the formation of both myeloid and erythroid colonies (Engstrom et al., 2003), suggesting that FoxO3 does plays an important role in lineage development.

Modulation of the activity of the PI3K signaling pathway has been observed to alter the level of reactive oxygen species (ROS). While ROS levels are reduced in PKBα/β deficient mice (Juntilla et al., 2010), increased levels have been observed in mice deficient for FoxO (Miyamoto et al., 2007). Increasing ROS levels in PKBα/β deficient mice was sufficient to rescue differentiation defects, but not impaired long-term hematopoiesis (Juntilla et al., 2010). Restoring the ROS levels in FoxO deficient mice by i*n vivo* treatment with an antioxidative agent N-acetyl-L-cysteine was sufficient to abrogate the enhanced levels of proliferation and apoptosis in FoxO deficient HSCs and to restore the reduced colony forming ability of these cells (Tothova et al., 2007). These studies demonstrate that correct regulation of ROS by FoxO transcription factors is essential for normal hematopoiesis.

Recent findings have demonstrated that correct regulation of the activity of GSK-3, another downstream effector of PKB, is also essential for maintenance of hematopoietic stem cell homeostasis. A reduction in long-term, but not short-term repopulating HSCs has, for example, been observed in GSK3 deficient mice (Huang et al., 2009). In addition, disruption of GSK-3 activity in mice with a pharmacological inhibitor or shRNAs has been shown to transiently induce expansion of both hematopoietic stem and progenitor cells followed by exhaustion of long-term repopulation HSCs (Trowbridge et al., 2006; Huang et al., 2009). In addition, since GSK-3 has been demonstrated to inhibit mTOR activity by phosphorylation and activation of TSC1/2 (Inoki et al., 2006) and the level of phosphorylated S6 was enhanced in cells with reduced GSK-3 levels, mice were treated with rapamycin. Rapamycin induced the number of LSK cells when GSK3 was depleted, but not in un-manipulated cells, suggesting that mTOR is an important effector of GSK-3 in regulation of HSC numbers (Huang et al., 2009) In addition to the observed expansion of HSCs in mice treated with a GSK-3 inhibitor, the recovery of neutrophil and megakaryocyte numbers after transplantation was accelerated in these mice, resulting in improved survival of the recipients (Trowbridge et al., 2006). In addition, *ex vivo* experiments revealed that GSK-3 can enhance eosinophil differentiation and inhibit neutrophil development (Buitenhuis et al., 2008). C/EBPα, a key regulator of hematopoiesis, has been demonstrated to be an important mediator of PKB/GSK-3 signaling in regulation of granulocyte development (Buitenhuis et al., 2008).

A third, important mediator of PI3K/PKB signaling is mTOR. Conditional deletion of TSC1 in mice, resulting in activation of mTOR, has been demonstrated to enhance the percentage of cycling HSCs and to reduce the self-renewal capacity of HSCs in serial transplantation assays (Chen et al., 2008). In addition, a reduction in the number of granulocytes and lymphocytes has been observed in those mice (Chen et al., 2008). As described above, activation of the PI3K signaling pathway by conditional deletion of PTEN in adult murine HSCs resulted in an initial expansion followed by exhaustion of LT-HSCs. Inhibition of mTOR in murine HSCs deficient for PTEN with Rapamycin was sufficient to revert this phenotype, again suggesting that mTORC1 signaling plays an important role in proliferation of HSCs (Yilmaz et al., 2006). A role for mTOR in progenitor expansion has been demonstrated utilizing an *ex vivo* human granulocyte differentiation system (Geest et al., 2009). In contrast to inhibition of PKB activity which not only affects progenitor expansion but also alters lineage development (Buitenhuis et al., 2008), inhibition of mTOR activity with Rapamycin only reduced the expansion of hematopoietic progenitors, during both eosinophil and neutrophil differentiation, without altering levels of apoptosis or maturation (Geest et al., 2009). Similarly, inhibition of mTOR reduced the number of interstitial DCs and Langerhans cells in *in vitro* experiments (van de Laar et al., 2010). In contrast to granulocyte development, treatment with rapamycin appears not only to affect proliferation during megakaryocyte (MK) development, but also appears to delay the generation of pro-platelet MKs (Raslova et al., 2006). Similar to FOXO transcription factors, TSC1 also appears to be involved in regulation of ROS levels in HSCs. Elevated levels of ROS have been observed in TSC1 deficient mice. *In vivo* treatment of those mice with a ROS antagonist restored HSC numbers and function (Chen et al., 2008), suggesting that TSC1 regulates HSC numbers at least in part via ROS. In addition to GSK3, the activity of C/EBPα also appears to be regulated by mTOR, albeit in a different manner. It has recently been shown that the ratio of wild type C/EBPα (C/EBPαp42) and truncated C/EBPαp30, which is generated by alternative translation initiation, is decreased by mTOR, resulting in high levels of the smaller p30 C/EBPα isoform (Fu et al., 2010) that inhibits trans-activation of C/EBPα target genes in a dominant-negative manner (Pabst et al., 2001) and binds to the promoters of a unique set of target genes to suppress their transcription (Wang et al., 2007).

3. PI3K/PKB signaling and malignant hematopoiesis

3.1 Deregulated PI3K/PKB signaling in malignant hematopoiesis

The above described studies clearly demonstrate that the PI3K/PKB signaling module plays a critical role in regulation of hematopoiesis. Since constitutive activation of PI3K and/or its downstream effectors has been observed in a high percentage of patients with hematological malignancies, it is likely that the development of leukemia may at least in part depend on aberrant regulation of this signaling module.

3.1.1 PI3K

Constitutive activation of class I PI3K isoforms has been observed in a high percentage of patients with acute leukemia (Kubota et al., 2004; Silva et al., 2008; Billottet et al., 2009; Zhao, 2010). In contrast to the expression of p110α, β and γ which is only up-regulated in leukemic blasts of some patients, p110δ expression appears to be consistently up-regulated in cells from patients with either AML or APL (Sujobert et al., 2005; Billottet et al., 2009). Activating

mutations in p110α, have been detected in a wide variety of human solid tumors (Ligresti et al., 2009). The most common mutations in p110α are located in the kinase domain (H1047R) and in the helical domain (E545A) (Lee et al., 2005). The E545A mutation has also been detected in acute, but not further specified, leukemia, albeit in a very low percentage (1/88) (Lee et al., 2005). In a series of 44 pediatric T-ALL patients, activating mutations in the catalytic subunit of PI3K (PIK3CA) have been observed in 2 patients, while in frame insertions/deletions have been detected in the PI3K regulatory subunit PIK3R1 in two other patients (Gutierrez et al., 2009). Transplantation of mice with bone marrow cells ectopically expressing mutated p110α resulted in the development of a leukemia-like disease within 5 weeks after transplantation (Horn et al., 2008), suggesting that mutations in p110α would be sufficient to induce leukemia. However, since mutations in PI3K appear to be very rare, it is unlikely that these mutations would be a major cause of leukemic development. Alternatively, the constitutive activation of PI3K observed in many patients with leukemia could also be caused by either aberrant expression or activation of modulators of PI3K activity, including PTEN and SHIP1.

Reduced expression of PTEN has, for example, been observed in different types of leukemia (Xu et al., 2003; Nyakern et al., 2006). Both homozygous and heterozygous deletion of PTEN as well as non-synonymous sequence alterations in exon 7 have been detected in approximately 15% and 25% of T-ALL patients, respectively (Gutierrez et al., 2009). In contrast, analysis of both leukemic cell lines and primary AML blasts indicate that PTEN mutations are rare in AML (Aggerholm et al., 2000; Liu et al., 2000). In addition to mutations in PTEN itself, aberrant PTEN expression may also be caused by mutations in its upstream regulators. Both enhanced casein kinase 2 (CK2) expression/activity and enhanced ROS levels appear, for example, to correlate with decreased PTEN phosphatase activity in T-ALL cells (Silva et al., 2008). Both CK2 inhibitors and ROS scavengers were sufficient to restore PTEN activity and impaired PI3K/PKB signaling in those T-ALL cells, demonstrating that aberrant CK2 and ROS levels may affect PI3K signaling in leukemia (Silva et al., 2008). Another important, negative regulator of PI3K activity that has been demonstrated to play a critical role in hematopoiesis is SHIP1. Analysis of primary T-ALL cells revealed that full length SHIP1 expression is often low or undetectable. However, when using an antibody against the C terminal domain of SHIP1, low molecular weight proteins can frequently be observed. These low molecular weight proteins are thought to be the result of mutation induced alternative splicing (Lo et al., 2009). In addition, in leukemic cells from an AML patient, a mutation in the phosphatase domain of SHIP1 has also been detected which results in reduced catalytic activity and enhanced PKB phosphorylation (Luo et al., 2003). For an overview of all known mutations affecting PI3K/PKB signaling, see table 1.

3.1.2 PKB

Constitutive activation of PKB has been demonstrated in a significant fraction of AML patients (Min et al., 2003; Xu et al., 2003; Zhao et al., 2004; Grandage et al., 2005; Gallay et al., 2009). Until recently, no PKB mutations were found in patients with leukemia. However, an activating mutation in the pleckstrin homology domain of PKB (E17K) has recently been detected in solid tumors (Carpten et al., 2007). Transplantation of mice with bone marrow cells ectopically expressing this E17K mutation was sufficient to induce leukemia, ten weeks after transplantation (Carpten et al., 2007). Although this particular mutation has been observed in different types of cancer, it appears to be rare in leukemic patients. Thus far, this

mutation has only been detected in one pediatric T-ALL patient (Gutierrez et al., 2009). To date, no other mutations in PKB have been described.

	Mutation	Activation/ loss	Detected in:	Location	References
Pathway					
PI3K	E545A	Activation	AML & ALL	Helical domain p110α	Lee , 2005; Horn , 2008
	E542K	Activation	#	Helical domain p110α	Horn , 2008
	H1047R	Activation	#	Kinase domain p110α	Horn , 2008
	PIK3CA	Activation	T-ALL	Catalytic subunit PI3K	Gutierrez , 2009
	PIK3R1	Deletion	T-ALL	Regulatory subunit PI3K	Gutierrez , 2009
PTEN	PTEN	Deletion	T-ALL	Homozygous and heterozygous	Gutierrez , 2009
		Dysruption	T-ALL	Sequence alterations in exon 7	Gutierrez , 2009
		Deletion	ALL cell line	Exons 2 through 5	Sakai , 1998
		Deletion	AMl cell line	Exons 2 through 5	Aggerholm , 2000
SHIP1	SHIP1	Deactivation	AML	Phosphatase domain	Luo , 2003
PKB	E17K	Activation	T-ALL	Pleckstrin homology domain	Carpten , 2007; Gutierrez , 2009
PP2A	Deletion	Deletion/Loss	AML		Cristobal , 2011
Upstream					
Flt3	Flt3-ITD	Activation	AML & ALL	Juxtamembrane (JM) domain	Reviewed by Parcells , 2006
	JM-point mutation	Less autoinhibition	AML	Juxtamembrane (JM) domain	
	AL-point mutation	Activation	AML & ALL	Activation loop (AL) of the kinase domain	
	K663Q	Activation	AML	First mutation outside JM and AL domain	
c-Kit	EC-point mutation	Activation	AML	Extracellular (EC) domain of the kinase	Yuzawa, 2007
	AL-point mutation	Activation	AML	Activation loop (AL) of the kinase domain	Reviewed by Scholl , 2008
Ras	Mutations	Activation	AML & ALL		Gutierrez , 2009; Dicker , 2010
Bcr-Abl	Translocation	Activation	ALL	t(9;22) (q34;q11)	Clark , 1988; Varticovski , 1991

Mutation induces leukemia in mouse model.

Table 1. Mutations in the PI3K/PKB pathway.

3.1.3 Activating mutations upstream of PI3K/PKB signaling pathway

The PI3K/PKB signaling module is an important mediator of cytokine signals. In hematological malignancies, mutations in cytokine receptors have been described to affect

PI3K signaling. Constitutive activation of FMS-like tyrosine kinase 3 (FLT3), by internal tandem duplication (Flt3-ITD) (Brandts et al., 2005) and mutation in c-Kit (Ning et al., 2001) have, for example, been demonstrated to induce PKB activity. This induction of PKB activity appears to be essential for the survival and proliferation of cells expressing FLT3-ITD (Brandts et al., 2005) or mutated c-Kit (Hashimoto et al., 2003; Cammenga et al., 2005; Horn et al., 2008). In addition to these tyrosine kinase receptors, the activity of the PI3K/PKB pathway can also be enhanced by several fusion proteins, including Bcr-Abl, which can be detected in virtually all patients with CML (Ben-Neriah et al., 1986) and in patients with ALL (Clark et al., 1988). It has been demonstrated that the PI3K/PKB signal transduction pathway plays an important role in Bcr-abl mediated leukemic transformation (Varticovski et al., 1991; Skorski et al., 1997; Hirano et al., 2009). Other potential regulators of PI3K often mutated in leukemia include Ras (Rodriguez-Viciana et al., 1994; reviewed by Schubbert et al., 2007; Gutierrez et al., 2009) Evi1 (Yoshimi et al., 2011) and PP2A. In AML patients, decreased PP2A activity has, for example, been reported to correlate with enhanced levels of PKB phosphorylation on Thr308 (Gallay et al., 2009). In addition, restoration of PP2A activity also resulted in a reduction of PKB phosphorylation (Cristobal et al., 2011).

3.2 Prognosis of acute leukemia with activated PI3K/PKB signaling

As described above, the PI3K/PKB signaling module appears to be aberrantly regulated in a large fraction of patients with leukemia. Recent evidence suggests that the level of PI3K/PKB activation in leukemic blasts could be used to predict the survival rate of patients. Comparison of pediatric T-ALL patients with either no mutations in PTEN, mono-allelic mutations or bi-allelic mutations revealed that the survival rate of patients positively correlates with the level of PTEN (Jotta et al., 2010). Similar observations were made in a different cohort of pediatric T-ALL patients, in which PTEN deletions correlated with early treatment failure in T-ALL (Gutierrez et al., 2009). These studies suggest that constitutive activation of PI3K and its downstream effectors reduces the survival rate of ALL patients. To determine whether the level of mTOR activity similarly correlates with reduced survival of ALL patients, mice were transplanted with blasts from pediatric de novo B cell progenitor ALL patients. In those experiments, a rapid induction of leukemia correlated with enhanced mTOR activity in the leukemic blasts (Meyer et al., 2011). In addition to ALL, constitutive activation of PI3K, as measured by enhanced FoxO3 expression or phosphorylation, is also considered to be an independent adverse prognostic factor in AML patients (Santamaria et al., 2009; Kornblau et al., 2010). In addition, a reduced survival rate has also been observed in AML patients displaying enhanced levels of phosphorylated, and therefore inactive, PTEN (Cheong et al., 2003) and phosphorylated PKB on Serine 473 (Kornblau et al., 2006) and Threonine 308 (Gallay et al., 2009). In contrast, Tamburini *et al.* suggest that PI3K activity, as was determined by analysis of the level of phosphorylation of PKB on Ser^{473}, positively correlates with the survival of AML patients (Tamburini et al., 2007). Although the short-term survival rate (within 12 months) appeared to be slightly lower in the group displaying high PKB phosphorylation compared to the group with low levels of phosphorylated PKB, both the long-term survival and relapse free survival were significantly enhanced (Tamburini et al., 2007). Except for this last study, all other studies suggest that enhanced PI3K/PKB activity correlates with reduced survival rate in both ALL and AML patients. The molecular mechanisms underlying this reduced prognosis are, thus far, incompletely understood. However, it has been demonstrated that AML blasts

displaying enhanced PI3K/PKB activation exhibit a reduced apoptotic response (Rosen et al., 2010) which might be due to positive regulation of the anti-apoptotic NF-kB pathway and negative regulation of the P53 pathway (Grandage et al., 2005).
In addition, since PI3K has been demonstrated to induce expression of the multidrug resistance-associated protein 1 (MRP1), a member of the ATP-binding cassette (ABC) membrane transporters that functions as a drug efflux pump (Tazzari, Cappellini et al. 2007), it could also be hypothesized that constitutive activation of this signaling module results in drug-resistance. The observation that high levels of MRP1 correlates with enhanced drug resistance of AML cells and poor prognosis supports this hypothesis (Legrand et al., 1999; Mahadevan & List, 2004).

3.3 PI3K/PKB signaling as therapeutic target in acute leukemia

3.3.1 PI3K inhibitors

Since aberrant regulation of PI3K and its downstream effectors has frequently been observed in leukemic cells and are known to play a critical role in normal hematopoiesis, these molecules are considered to be promising targets for therapy (Table 2). Wortmannin and LY294002 are two well characterized inhibitors of PI3K activity that prevent ATP to bind to and activate PI3K by association with its catalytic subunit (Vlahos et al., 1994; Wymann et al., 1996). Although pre-clinical experiments indicate that both LY294002 and Wortmannin are potent inhibitors of PI3K activity, induce apoptosis in leukemic cells (Xu et al., 2003; Zhao et al., 2004) and rescue drug sensitivity (Neri et al., 2003), it has been demonstrated that both inhibitors exhibit little specificity within the PI3K family and can also inhibit other kinases, including CK2 and smMLCK, respectively (Davies et al., 2000; Gharbi et al., 2007). Since both inhibitors are also insoluble in an aqueous solution (Garlich et al., 2008; Zask et al., 2008) and are detrimental for normal cells (Gunther et al., 1989; Buitenhuis et al., 2008), different PI3K inhibitors are currently developed. Recently, while screening for inhibitors of Cyclin D expression, a novel inhibitor of PI3K activity (S14161) has been discovered that appears to be able to delay tumor growth in mice transplanted with human leukemic cell lines (Mao et al., 2011). In addition, novel inhibitors have been developed that efficiently block the activity of individual p110 isoforms. The p110δ-selective inhibitor IC87114, for example, significantly reduced proliferation and survival of AML blasts (Sujobert et al., 2005) and APL cells (Billottet et al., 2009) without affecting the proliferation of normal hematopoietic progenitors (Sujobert et al., 2005). Similar results were obtained in APL cells treated with an inhibitor directed against p110β (TGX-115) (Billottet et al., 2009).

3.3.2 PKB inhibitors

In addition to PI3K inhibitors, research has also focused on the development of pharmacological compounds that inhibit its downstream effector PKB. Perifosine, a synthetic alkylphosphocholine with oral bioavailability inhibits PKB phosphorylation by competitive interaction with its PH doma1in (Kondapaka et al., 2003) and promotes degradation of PKB, mTOR, Raptor, Rictor, p70S6K and 4E-BP1 (Fu et al., 2009). *In vitro* experiments with multidrug-resistant human T-ALL cells and primary AML cells revealed that treatment with Perisofine is sufficient to induce apoptosis (Chiarini et al., 2008; Papa et al., 2008). Moreover, Perifosine reduced the clonogenic activity of AML blasts, but not normal $CD34^+$ hematopoietic progenitor cells (Papa et al., 2008). The efficacy of Perifosine in treatment of different types of leukemia is currently examined in several phase II clinical

trials (NCT00391560, NCT00873457). Phosphatidylinositol ether lipid analogues (PIA) inhibit PKB activity in a similar manner compared to Perifosine. Treatment of HL60 cells with PIA resulted in inhibition of proliferation and sensitization to chemotherapeutic agents in concentrations which did not affect proliferation of normal hematopoietic progenitors (Tabellini et al., 2004). Another specific PKB inhibitor (AKT-I-1/2 inhibitor) (Bain et al., 2007), has been demonstrated to efficiently reduce colony formation in high-risk AML samples (Gallay et al., 2009). The PKB inhibitor Triciribine (API-2), a purine analog that has initially been identified as an inhibitor of DNA synthesis, inhibits PKB phosphorylation by interacting with the PH domain of PKB, thus preventing PKB membrane localization and phosphorylation (Berndt et al., 2010). Experiments in T-ALL cell lines revealed that API-2 induces cell cycle arrest and caspase-dependent apoptosis (Evangelisti et al., 2011a). The safety of this inhibitor is currently under investigation in a phase I clinical trial in patients with advanced hematologic malignancies (NCT00363454).

3.3.3 mTOR inhibitors

Rapamycin and its analogues RAD001 (everolimus), CCI-779 (temsirolimus) and AP23573 (deforolimus) inhibit the mTORC1 complex by association with FKBP-12 which prohibits association of Raptor with mTOR. (Choi et al., 1996; Oshiro et al., 2004). The efficacy of these compounds as therapeutic drugs has been examined in various preclinical and clinical studies for a wide range of malignancies (reviewed by Yuan et al., 2009; reviewed by Chapuis et al., 2010a). The anti-tumor properties of Rapamycin have also been examined in both AML derived cell lines and primary AML blasts, revealing a strong anti-tumor effect of this agent in short-term cultures (Recher et al., 2005). Furthermore, Rapamycin and its analog CCI-779 showed promising effects in preclinical models of T-ALL (Teachey et al., 2008; Meyer et al., 2011) and pre-B ALL (Teachey et al., 2006), respectively. Clinical trials initiated to examine the efficacy of Rapamycin (Recher et al., 2005) and its analog AP23573 in hematological malignancies only resulted in a partial response (Rizzieri et al., 2008). The limited therapeutic effects of Rapamycin and AP23573 may be explained by the induction of PKB activity in AML blasts treated with these compounds (Easton & Houghton, 2006; Tamburini et al., 2008; Yap et al., 2008). Furthermore, experiments with PTEN deficient mice revealed that, due to failure to eliminate the leukemic stem cell population, withdrawal of rapamycin results in a rapid re-induction of leukemia and death in the majority of mice (Guo et al., 2011). This suggests that rapamycin primarily has cytostatic, but not cytotoxic, effects on hematopoietic stem cells.

To circumvent the observed up-regulation of PKB phosphorylation by Rapamycin and its analogs, ATP-competitive mTOR inhibitors have been generated that inhibit both the activity of mTORC1 and mTORC2 (Garcia-Martinez et al., 2009; Bhagwat & Crew, 2010; Janes et al., 2010). Treatment of mice transplanted with primary ALL blasts or pre-leukemic thymocytes over-expressing PKB with the mTORC 1/2 inhibitor PP242, but not Rapamycin, significantly reduced the development of leukemia (Hsieh et al., 2010; Janes et al., 2010). Importantly, PP242 appears to induce less adverse effects on proliferation and function of normal lymphocytes in comparison to Rapamycin (Janes et al., 2010; Evangelisti et al., 2011b). In addition to PP-242, another mTORC1/2 inhibitor, OSI-027, has recently been described. (Evangelisti et al., 2011). It has been demonstrated that this inhibitor exhibits anti-leukemic effects in both Ph+ ALL and CML cells (Carayol et al., 2010). Furthermore, proliferation experiments indicate that, in comparison to Rapamycin, OSI-027 is a more efficient suppressor of proliferation of AML cell lines (Altman et al., 2011).

3.3.4 Dual inhibition of the PI3K/PKB pathway

In addition to the recently developed mTORC1/2 inhibitors, dual specificity inhibitors have been generated to further optimize inhibition of the PI3K signaling module. PI-103, a synthetic small molecule of the pyridofuropyrimidine class is, for example, a potent inhibitor for both class I PI3K isoforms and mTORC1 (Raynaud et al., 2007). PI-103 has been demonstrated to reduce proliferation and survival of cells from T-ALL (Chiarini et al., 2009) and AML patients (Kojima et al., 2008; Park et al., 2008) and appears to exhibit a stronger anti-leukemic activity compared to both Rapamycin (Chiarini et al., 2009) and the combination of RAD001 and IC87114 (Park et al., 2008). Importantly, although PI-103 reduces proliferation of normal hematopoietic progenitors, survival is not affected (Park et al., 2008). Recently, NVP-BEZ235, another dual PI3K/mTOR inhibitor has been identified. This orally bioavailable imidazoquinoline derivative, has been demonstrated to inhibit the activity of both PI3K and mTOR by binding to their ATP-binding pocket (Maira et al., 2008). In both primary T-ALL (Chiarini et al., 2010) and AML cells (Chapuis, Tamburini et al. 2010b) as well as leukemic cell lines, NVP-BEZ235 significantly reduced proliferation and survival (Chapuis et al., 2010b; Chiarini et al., 2010). Furthermore, this compound did not affect the clonogenic capacity of normal hematopoietic progenitors (Chapuis et al., 2010b). A dual PI3K/PDK1 inhibitor called BAG956 has also recently been described to inhibit proliferation of BCR-ABL and FLT3-ITD expressing cells. However, in contrast to RAD001 which efficiently reduced the tumor load in mice transplanted with BCR-ABL expressing cells, treatment with BAG956 alone was not sufficient to reduce the tumor load (Weisberg et al., 2008). In addition to these dual inhibitors, KP372-1, a multiple kinase inhibitor capable of inhibiting PKB, PDK1, and FLT3 has been described (Zeng et al., 2006). It has been demonstrated that KP372-1 can induce apoptosis in primary AML cells and leukemic cell lines, as was visualized by mitochondrial depolarization and phosphatidylserine externalization (Zeng et al., 2006). Although the survival of normal hematopoietic progenitors was not impaired by this compound, their clonogenic capacity was, albeit with a low efficiency (Zeng et al., 2006).

In addition to the above described dual inhibitors, the efficacy of combination therapy utilizing multiple inhibitors, which are directed against different intermediates of the PI3K signaling module, is also under investigation. To abrogate the RAD001 mediated up-regulation of PKB phosphorylation, the p110δ inhibitor IC87114 has, for example, been added to leukemic cells simultaneously with RAD001. Combined inhibition of mTOR and p110δ not only resulted in a block in PKB phosphorylation in primary AML blasts, but a synergistic reduction in proliferation could also be observed (Tamburini et al., 2008). Similarly, combining the PI3K/PDK1 inhibitor BAG956 with RAD001 also resulted in a synergistic reduction in tumor volume in a mouse model transplanted with BCR-ABL expressing cells (Weisberg et al., 2008). Recently, a phase I trial focusing on development of a combination regimen including both perifosine and UCN-01 (NCT00301938), a PDK1 inhibitor which is known to induce apoptosis in AML cells *in vitro* (Hahn et al., 2005), has been initiated.

3.3.5 Combination of PI3K/PKB pathway inhibitors with other pathway inhibitors

Leukemogenesis involves aberrant regulation of various signal transduction pathways, including, but not limited to, the PI3K signaling module. Simultaneous targeting of multiple

Target	Compound	Effect		Clinical Trials (phase)	Leukemia	References
		In vitro	*In vivo*			
PI3K	Wortmannin	+	-	-		Wymann , 1996
	LY294002	+	-	-		Xu , 2003; Zhao , 2004
	S14161	+	+	-		Mao , 2011
p110β	TGX-115	+	-	-		Billottet , 2009
p110δ	IC87114	+	-	-		Sujobert , 2005; Billottet , 2006, 2009
	AMG 319	-	-	NCT01300026 (I)	ALL	
PDK1	UCN-01	+	-	-		Hahn , 2005
PKB	Perifosine	+	-	NCT00391560 (II)	AML&ALL	Chiarini , 2008; Fu , 2009; Papa , 2008
				NCT00873457 (II)	CLL	
	PIA	+	-	-		Tabellini , 2004
	AKT-I-1/2	+	-	-		Gallay , 2009
	Triciribine (API-2)	+	-	NCT00363454 (I)	-	Evangelisti , 2011a
	GSK690693	+	-	NCT00666081 (I)	AML&ALL	Levy , 2009
	MK2206	-	-	NCT01231919 (I)	AML&ALL	
				NCT01253447 (II)	AML	
	SR13668	-	-	NCT00896207 (I)	-	
	GSK2141795	-	-	NCT00920257 (I)	-	
	GSK2111018 3	-	-	NCT00881946 (I/II)	AML&ALL	
mTOR	Rapamycin	+	+	NCT00795886 (I)	ALL	Recher 2005; Meyer , 2011; Teachey , 2008; Gu , 2010; Guo , 2011
	RAD001	+	+	Yee, 2006 (I/II)	AML	Yee , 2006
	CCI-779	+	+	Recher, 2005 (II)	AML	Teachey , 2006; Recher, 2005
	AP23573	-	-	Rizzieri, 2008 (II)	AML	Rizzieri, 2008
				NCT00086125 (II)	AML&ALL	
	PP242	+	+	-		Hsieh , 2010; Janes , 2010; Evangelisti , 2011b
	OSI-027	+	-	-		Evangelisti, 2011b ; Carayol, 2010; Altman, 2011
	AZD-8050	+	-	-	-	Evangelisti , 2011b
PI3K/mTOR	PI-103	+	+	-	-	Chiarini , 2009; Kojima , 2008; Park , 2008
PI3K/mTOR	NVP-BEZ235	+	+	-	-	Maira , 2008; Chiarini , 2010; Chapuis , 2010b
PI3K/PDK1	BAG956	+	+	-	-	Weisberg , 2008
PKB/PDK1/Flt3	KP372-1	+	-	-	-	Zeng , 2006

Table 2. Inhibitors of PI3K/PKB signaling pathway

aberrantly regulated signal transduction pathways is considered to be a promising therapeutic strategy (Table 3). Proteosome inhibitors are considered to be a new class of therapeutic agents. However, treatment of both pediatric and adult B-ALL patients with such an inhibitor (Bortezomib) alone was not sufficient to induce a robust anti-tumor response (Cortes et al., 2004; Horton et al., 2007). Experiments in leukemic cell lines and primary cells from B-ALL patients revealed that while MG132, a proteosome inhibitor, and RAD001 alone only modestly reduce cell viability, combined inhibition of proteosomes and mTOR significantly enhanced cell death (Saunders et al., 2011), suggesting a synergistic effect of both inhibitors. In addition to proteosome inhibitors, HDAC inhibitors have also emerged as a promising class of anti-tumor agents (reviewed by Minucci & Pelicci, 2006). Although the HDAC inhibitor MS-275 appears to induce growth arrest, apoptosis and differentiation of leukemic cell lines, in mouse models only a partial reduction in tumor volume could be observed (Nishioka et al., 2008). Combined administration of MS-275 and RAD001, however, potentiated the effect of both inhibitors individually both *in vitro* and *in vivo* (Nishioka et al., 2008). Synergistic effects on proliferation and survival of leukemic cell lines have also been observed after co-administration of HDAC inhibitors and the PKB inhibitor Perisofine (Rahmani et al., 2005). Additionally, the efficacy of specific inhibitors targeting constitutively activated tyrosine kinases in leukemia, including inhibitors of Flt3, Abl, and c-Kit, has been investigated in preclinical and clinical models. Although anti-leukemia effects were observed *in vivo* and *in vitro*, combined inhibition of tyrosine kinases and the PI3K/PKB pathway resulted in a synergistically enhanced anti-leukemia effect in ALL (Kharas et al., 2008; Weisberg et al., 2008) and AML (Weisberg et al., 2008) compared to the individual inhibitors. Phase I/II clinical trials have already been initiated to investigate the synergistic effects of combined inhibition of PI3K/PKB and Flt3 (NCT00819546) or c-Kit (NCT00762632).

3.3.6 Combination of PI3K/PKB pathway inhibitors with chemotherapeutical agents

Despite the effectiveness of chemotherapy in a subset of patients, incomplete remission and the development of a refractory disease have been observed in many patients with acute leukemia (Thomas, 2009; Burnett et al., 2011). To optimize treatment of those patients, chemotherapy could potentially be combined with leukemia-specific inhibitors or chemosensitizing drugs (Table 3). Co-administration of mTOR inhibitors with different types of chemotherapeutic drugs, including Etoposide, Ara-C, Cytarabine and Dexamethason has, for example, been demonstrated to induce synergistic anti-leukemia effects in cells from AML patients (Xu et al., 2003; Xu et al., 2005) and ALL patients (Avellino et al., 2005; Teachey et al., 2008; Bonapace et al., 2010; Gu et al., 2010; Saunders et al., 2011). Several phase I/II clinical trials have been initiated to investigate and optimize the synergistic effect of mTOR inhibitors and chemotherapeutic drugs in patients with acute leukemia (NCT00544999, NCT01184898, NCT00780104, NCT01162551 and NCT00776373). In addition, co-administration of chemotherapeutic agents with IC87114 (Billottet et al., 2006), UCN-01 (Sampath et al., 2006) or Triciribine (Evangelisti et al., 2011a) showed similar synergistic effects in AML cells. Strong synergistic, cytotoxic, activity was also observed in T-ALL cells when combining the dual specificity inhibitors PI-103 and NVP-BEZ235 with chemotherapy (Chiarini et al., 2009; Chiarini et al., 2010).

Target	Compound	Combination regimens	Effects *in vitro/ in vivo*		Clinical trials (phase)	Leukemia	References
PI3K	Wortmannin	ATRA (DA)	+	-	-		Neri , 2003
	LY294002	Apigenin (CK2 I)	+	-	-		Cheong , 2010
		ATRA (DA)	+	-	-		Neri , 2003
p110δ	IC87114	VP16 (CT)	+	-	-		Billottet , 2006
PDK1	UCN-01	Ara-c (CT)	+	-	Sampath, 2006 (II)	AML	Sampath , 2006
		Cytarabine (CT)	-	-	NCT00004263 (I)	AML	
		Fludarabine (CT)	-	-	NCT00019838 (I)	AML&ALL	
PP2A	Forskolin	Idarubicine/Ara-C	+	-	-		Cristobal , 2011
PKB	Perifosine	UCN-01	-	-	NCT00301938 (I)	AML&ALL	
		HDAC I	+	-	-		Rahmani , 2005
		TRAIL (AI)	+	-	-		Tazzari , 2008
		Etoposide (CT	+	-	-		Papa , 2008
	PIA	CT	+	-	-		Tabellini , 2004
	Triciribine	Cytarabine (CT)	+	-	-		Evangelisti , 2011a
mTOR	Rapamycin	UCN-01	+	-	-		Hahn , 2005
		3-BrOP (glycolysis I)	+	-	-		Akers , 2011
		Notch I	+	-	-		Chan , 2007
		Dexamethason	+	-	-		Gu, 2010; Bonapace , 2010
		Etoposide (CT)	+	+	-		Xu , 2005
		Methotrexate (CT)	+	+	NCT01162551 (II)	ALL	Teachey , 2008
		Anthracyclin (CT)	+	-	-		Avellino , 2005
		CT	+	-	NCT00776373 (I/II)	ALL	
			+	-	NCT01184898 (I/II)	AML	
					NCT00780104 (I/II)	AML	
	RAD001	IC87114	+	-	-		Tamburini , 2008
		BAG956	+	+	-		Weisberg , 2008
		Bortezomib (PI)	+	-	-		Saunders , 2011
		MS-275 (HDAC I)	+	+	-		Nishioka , 2008
		PKC412 (Flt3 TKI)	-	-	NCT00819546 (I)	AML	
		Nilotinib (c-Kit-TKI)	-	-	NCT00762632 (I/II)	AML	
		ATRA (DA)	+	+	-		Nishioko , 2009
		Ara-c (CT)	+	-	-		Xu , 2003; Saunders , 2011
		Vincristine (CT)	+	-	-	ALL	Crazzolara, 2009
		CT	+	-	NCT00544999 (I)	AML&ALL	
	CCI-779	Methotrexate (CT)	+	+	-		Teachey , 2008
	PP242	Vincristine (CT)	+	-			Evangelisti , 2011b
PI3K/mTOR	PI-103	Nutlin-3 (MDM2-I)	+	-			Kojima , 2008
		Vincristine (CT)	+	-			Chiarini , 2009
		Imatinib (Bcr-Abl-TKI)	+	-			Kharas , 2008
PI3K/mTOR	NVP-BEZ235	CT	+	-			Chiarini , 2010
PI3K/PDK1	BAG956	Imatinib (Bcr-Abl-TKI)	+	+			Weisberg , 2008
		PKC412 (Flt3 TKI)	+	+			Weisberg , 2008

DA: Differentiating agents; I: Inhibitor; CT: Chemotherapy; AI: Apoptosis inducer; PI: Proteasome inhibitor; TKI: Tyrosine kinase inhibitor.

Table 3. Combination regimens.

4. Conclusion

During the last two decades, it has become clear that intracellular signal transduction pathways play an important role in both normal and malignant hematopoiesis. One such module implicated in playing a critical role in regulation of various hematopoietic processes includes PI3K and PKB. Aberrant regulation of these molecules appears to be sufficient to induce hematological malignancies. As discussed in this chapter, constitutive activation of this signaling module has been observed in a large group of acute leukemia's. Although activating mutations in PI3K and PKB have been detected in cells from patients with leukemia, these mutations appear to be very rare. In patients, mutations have also been observed in PTEN and SHIP1 resulting in activation of PI3K and its downstream effectors. These mutations, however, cannot account for the large incidence of constitutive activation of PI3K in patients with leukemia. Alternatively, constitutive activation of PI3K and PKB can also be induced by mutations in, for example, tyrosine kinase receptors and by translocation induced formation of fusion proteins. Since PI3K is frequently activated in leukemia and activation of this molecule is thought to correlate with poor prognosis and drug resistance, it is considered to be a promising target for therapy. A high number of pharmacological inhibitors directed against both individual and multiple components of this pathway has already been developed in order to improve therapy. Especially the dual specificity inhibitors seem to possess promising anti-leukemic activities. In addition, research currently focuses on combining inhibitors of the PI3K signaling module with either inhibitors directed against other signal transduction molecules or classic chemotherapy. Mouse models and in vitro experiments indicate that both strategies could be used to improve current therapeutic regimes in specific patient groups. To confirm the pre-clinical data and to examine the safety and efficacy of the individual inhibitors and combination regimes in patients with leukemia, several phase I and II clinical trials have already been initiated.

5. Acknowledgements

R. Polak was supported by a grant from KiKa (Children Cancer free).

6. References

Aggerholm, A.,K. Gronbaek, et al. (2000). Mutational analysis of the tumour suppressor gene MMAC1/PTEN in malignant myeloid disorders. *European Journal of Haematology*, Vol.65, No.2, pp. 109-113, ISSN 0902-4441

Akers, L. J.,W. Fang, et al. (2011). Targeting glycolysis in leukemia: A novel inhibitor 3-BrOP in combination with rapamycin. *Leukemia Research*, pp. ISSN 1873-5835

Altman, J. K.,A. Sassano, et al. (2011). Dual mTORC2/mTORC1 targeting results in potent suppressive effects on acute myeloid leukemia (AML) progenitors. *Clinical Cancer Research*, pp. ISSN 1078-0432

Avellino, R.,S. Romano, et al. (2005). Rapamycin stimulates apoptosis of childhood acute lymphoblastic leukemia cells. *Blood*, Vol.106, No.4, pp. 1400-1406, ISSN 0006-4971

Bain, J.,L. Plater, et al. (2007). The selectivity of protein kinase inhibitors: a further update. *The Biochemical Journal*, Vol.408, No.3, pp. 297-315, ISSN 1470-8728

Ben-Neriah, Y.,G. Q. Daley, et al. (1986). The chronic myelogenous leukemia-specific P210 protein is the product of the bcr/abl hybrid gene. *Science*, Vol.233, No.4760, pp. 212-214, ISSN 0036-8075

Berndt, N.,H. Yang, et al. (2010). The Akt activation inhibitor TCN-P inhibits Akt phosphorylation by binding to the PH domain of Akt and blocking its recruitment to the plasma membrane. *Cell Death and Differentiation*, Vol.17, No.11, pp. 1795-1804, ISSN 1476-5403

Bhagwat, S. V. and A. P. Crew. (2010). Novel inhibitors of mTORC1 and mTORC2. *Current Opinion in Investigational Drugs*, Vol.11, No.6, pp. 638-645, ISSN 2040-3429

Billottet, C.,L. Banerjee, et al. (2009). Inhibition of class I phosphoinositide 3-kinase activity impairs proliferation and triggers apoptosis in acute promyelocytic leukemia without affecting atra-induced differentiation. *Cancer Research*, Vol.69, No.3, pp. 1027-1036, ISSN 1538-7445

Billottet, C.,V. L. Grandage, et al. (2006). A selective inhibitor of the p110delta isoform of PI 3-kinase inhibits AML cell proliferation and survival and increases the cytotoxic effects of VP16. *Oncogene*, Vol.25, No.50, pp. 6648-6659, ISSN 0950-9232

Birkenkamp, K. U. and P. J. Coffer. (2003). FOXO transcription factors as regulators of immune homeostasis: molecules to die for? *Journal of Immunology*, Vol.171, No.4, pp. 1623-1629, ISSN 0022-1767

Bonapace, L.,B. C. Bornhauser, et al. (2010). Induction of autophagy-dependent necroptosis is required for childhood acute lymphoblastic leukemia cells to overcome glucocorticoid resistance. *Journal of Clinical Investigation*, Vol.120, No.4, pp. 1310-1323, ISSN 1558-8238

Brandts, C. H.,B. Sargin, et al. (2005). Constitutive activation of Akt by Flt3 internal tandem duplications is necessary for increased survival, proliferation, and myeloid transformation. *Cancer Research*, Vol.65, No.21, pp. 9643-9650, ISSN 0008-5472

Brunet, A.,A. Bonni, et al. (1999). Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. *Cell*, Vol.96, No.6, pp. 857-868, ISSN 0092-8674

Buitenhuis, M.,L. P. Verhagen, et al. (2008). Protein kinase B (c-akt) regulates hematopoietic lineage choice decisions during myelopoiesis. *Blood*, Vol.111, No.1, pp. 112-121, ISSN 0006-4971

Burgering, B. M. and P. J. Coffer. (1995). Protein kinase B (c-Akt) in phosphatidylinositol-3-OH kinase signal transduction. *Nature*, Vol.376, No.6541, pp. 599-602, ISSN 0028-0836

Burnett, A.,M. Wetzler, et al. (2011). Therapeutic advances in acute myeloid leukemia. *Journal of Clinical Oncology*, Vol.29, No.5, pp. 487-494, ISSN 1527-7755

Calamito, M.,M. M. Juntilla, et al. (2010). Akt1 and Akt2 promote peripheral B-cell maturation and survival. *Blood*, Vol.115, No.20, pp. 4043-4050, ISSN 1528-0020

Cammenga, J.,S. Horn, et al. (2005). Extracellular KIT receptor mutants, commonly found in core binding factor AML, are constitutively active and respond to imatinib mesylate. *Blood*, Vol.106, No.12, pp. 3958-3961, ISSN 0006-4971

Carayol, N.,E. Vakana, et al. (2010). Critical roles for mTORC2- and rapamycin-insensitive mTORC1-complexes in growth and survival of BCR-ABL-expressing leukemic cells. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.107, No.28, pp. 12469-12474, ISSN 1091-6490

Carpten, J. D.,A. L. Faber, et al. (2007). A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. *Nature*, Vol.448, No.7152, pp. 439-444, ISSN 1476-4687

Chan, S. M.,A. P. Weng, et al. (2007). Notch signals positively regulate activity of the mTOR pathway in T-cell acute lymphoblastic leukemia. *Blood*, Vol.110, No.1, pp. 278-286, ISSN 0006-4971

Chapuis, N.,J. Tamburini, et al. (2010)a. Perspectives on inhibiting mTOR as a future treatment strategy for hematological malignancies. *Leukemia*, Vol.24, No.10, pp. 1686-1699, ISSN 1476-5551

Chapuis, N.,J. Tamburini, et al. (2010)b. Dual inhibition of PI3K and mTORC1/2 signaling by NVP-BEZ235 as a new therapeutic strategy for acute myeloid leukemia. *Clinical Cancer Research*, Vol.16, No.22, pp. 5424-5435, ISSN 1078-0432

Chen, C.,Y. Liu, et al. (2008). TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. *The Journal of Experimental Medicine*, Vol.205, No.10, pp. 2397-2408, ISSN 1540-9538

Cheong, J. W.,J. I. Eom, et al. (2003). Phosphatase and tensin homologue phosphorylation in the C-terminal regulatory domain is frequently observed in acute myeloid leukaemia and associated with poor clinical outcome. *British Journal of Haematology*, Vol.122, No.3, pp. 454-456, ISSN 0007-1048

Cheong, J. W.,Y. H. Min, et al. (2010). Inhibition of CK2{alpha} and PI3K/Akt synergistically induces apoptosis of CD34+CD38- leukaemia cells while sparing haematopoietic stem cells. *Anticancer Research*, Vol.30, No.11, pp. 4625-4634, ISSN 1791-7530

Chiarini, F.,M. Del Sole, et al. (2008). The novel Akt inhibitor, perifosine, induces caspase-dependent apoptosis and downregulates P-glycoprotein expression in multidrug-resistant human T-acute leukemia cells by a JNK-dependent mechanism. *Leukemia*, Vol.22, No.6, pp. 1106-1116, ISSN 1476-5551

Chiarini, F.,F. Fala, et al. (2009). Dual inhibition of class IA phosphatidylinositol 3-kinase and mammalian target of rapamycin as a new therapeutic option for T-cell acute lymphoblastic leukemia. *Cancer Research*, Vol.69, No.8, pp. 3520-3528, ISSN 1538-7445

Chiarini, F.,C. Grimaldi, et al. (2010). Activity of the novel dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor NVP-BEZ235 against T-cell acute lymphoblastic leukemia. *Cancer Research*, Vol.70, No.20, pp. 8097-8107, ISSN 1538-7445

Choi, J.,J. Chen, et al. (1996). Structure of the FKBP12-rapamycin complex interacting with the binding domain of human FRAP. *Science*, Vol.273, No.5272, pp. 239-242, ISSN 0036-8075

Clark, S. S.,J. McLaughlin, et al. (1988). Expression of a distinctive BCR-ABL oncogene in Ph1-positive acute lymphocytic leukemia (ALL). *Science*, Vol.239, No.4841 Pt 1, pp. 775-777, ISSN 0036-8075

Cortes, J.,D. Thomas, et al. (2004). Phase I study of bortezomib in refractory or relapsed acute leukemias. *Clinical Cancer Research*, Vol.10, No.10, pp. 3371-3376, ISSN 1078-0432

Crazzolara, R.,A. Cisterne, et al. (2009). Potentiating effects of RAD001 (Everolimus) on vincristine therapy in childhood acute lymphoblastic leukemia. *Blood*, Vol.113, No.14, pp. 3297-3306, ISSN 1528-0020

Cristobal, I.,L. Garcia-Orti, et al. (2011). PP2A impaired activity is a common event in acute myeloid leukemia and its activation by forskolin has a potent anti-leukemic effect. *Leukemia*, pp. ISSN 1476-5551

Cross, D. A.,D. R. Alessi, et al. (1995). Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. *Nature*, Vol.378, No.6559, pp. 785-789, ISSN 0028-0836

Damen, J. E.,L. Liu, et al. (1996). The 145-kDa protein induced to associate with Shc by multiple cytokines is an inositol tetraphosphate and phosphatidylinositol 3,4,5-triphosphate 5-phosphatase. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.93, No.4, pp. 1689-1693, ISSN 0027-8424

Davies, S. P.,H. Reddy, et al. (2000). Specificity and mechanism of action of some commonly used protein kinase inhibitors. *The Biochemical Journal*, Vol.351, No.Pt 1, pp. 95-105, ISSN 0264-6021

Dowler, S.,R. A. Currie, et al. (2000). Identification of pleckstrin-homology-domain-containing proteins with novel phosphoinositide-binding specificities. *The Biochemical Journal*, Vol.351, No.Pt 1, pp. 19-31, ISSN 0264-6021

Easton, J. B. and P. J. Houghton. (2006). mTOR and cancer therapy. *Oncogene*, Vol.25, No.48, pp. 6436-6446, ISSN 0950-9232

Engstrom, M.,R. Karlsson, et al. (2003). Inactivation of the forkhead transcription factor FoxO3 is essential for PKB-mediated survival of hematopoietic progenitor cells by kit ligand. *Experimental Hematology*, Vol.31, No.4, pp. 316-323, ISSN 0301-472X

Evangelisti, C.,F. Ricci, et al. (2011)a. Preclinical testing of the Akt inhibitor triciribine in T-cell acute lymphoblastic leukemia. *Journal of Cellular Physiology*, Vol.226, No.3, pp. 822-831, ISSN 1097-4652

Evangelisti, C.,F. Ricci, et al. (2011)b. Targeted inhibition of mTORC1 and mTORC2 by active-site mTOR inhibitors has cytotoxic effects in T-cell acute lymphoblastic leukemia. *Leukemia*, pp. ISSN 1476-5551

Fruman, D. A.,F. Mauvais-Jarvis, et al. (2000). Hypoglycaemia, liver necrosis and perinatal death in mice lacking all isoforms of phosphoinositide 3-kinase p85 alpha. *Nature Genetics*, Vol.26, No.3, pp. 379-382, ISSN 1061-4036

Fu, C. T.,K. Y. Zhu, et al. (2010). An evolutionarily conserved PTEN-C/EBPalpha-CTNNA1 axis controls myeloid development and transformation. *Blood*, Vol.115, No.23, pp. 4715-4724, ISSN 1528-0020

Fu, L.,Y. A. Kim, et al. (2009). Perifosine inhibits mammalian target of rapamycin signaling through facilitating degradation of major components in the mTOR axis and induces autophagy. *Cancer Research*, Vol.69, No.23, pp. 8967-8976, ISSN 1538-7445

Gallay, N.,C. Dos Santos, et al. (2009). The level of AKT phosphorylation on threonine 308 but not on serine 473 is associated with high-risk cytogenetics and predicts poor overall survival in acute myeloid leukaemia. *Leukemia*, Vol.23, No.6, pp. 1029-1038, ISSN 1476-5551

Garcia-Martinez, J. M.,J. Moran, et al. (2009). Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). *The Biochemical Journal*, Vol.421, No.1, pp. 29-42, ISSN 1470-8728

Garlich, J. R.,P. De, et al. (2008). A vascular targeted pan phosphoinositide 3-kinase inhibitor prodrug, SF1126, with antitumor and antiangiogenic activity. *Cancer Research*, Vol.68, No.1, pp. 206-215, ISSN 1538-7445

Geest, C. R.,F. J. Zwartkruis, et al. (2009). Mammalian target of rapamycin activity is required for expansion of CD34+ hematopoietic progenitor cells. *Haematologica*, Vol.94, No.7, pp. 901-910, ISSN 1592-8721

Gharbi, S. I.,M. J. Zvelebil, et al. (2007). Exploring the specificity of the PI3K family inhibitor LY294002. *The Biochemical Journal*, Vol.404, No.1, pp. 15-21, ISSN 1470-8728

Golub, T. and P. Caroni. (2005). PI(4,5)P2-dependent microdomain assemblies capture microtubules to promote and control leading edge motility. *Journal of Cell Biology*, Vol.169, No.1, pp. 151-165, ISSN 0021-9525

Grandage, V. L.,R. E. Gale, et al. (2005). PI3-kinase/Akt is constitutively active in primary acute myeloid leukaemia cells and regulates survival and chemoresistance via NF-kappaB, Mapkinase and p53 pathways. *Leukemia*, Vol.19, No.4, pp. 586-594, ISSN 0887-6924

Gu, L.,C. Zhou, et al. (2010). Rapamycin sensitizes T-ALL cells to dexamethasone-induced apoptosis. *Journal of Experimental & Clinical Cancer Research*, Vol.29, pp. 150, ISSN 1756-9966

Gunther, R.,H. K. Abbas, et al. (1989). Acute pathological effects on rats of orally administered wortmannin-containing preparations and purified wortmannin from Fusarium oxysporum. *Food and Chemical Toxicology*, Vol.27, No.3, pp. 173-179, ISSN 0278-6915

Guo, W.,S. Schubbert, et al. (2011). Suppression of leukemia development caused by PTEN loss. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.108, No.4, pp. 1409-1414, ISSN 1091-6490

Gutierrez, A.,T. Sanda, et al. (2009). High frequency of PTEN, PI3K, and AKT abnormalities in T-cell acute lymphoblastic leukemia. *Blood*, Vol.114, No.3, pp. 647-650, ISSN 1528-0020

Hahn, M.,W. Li, et al. (2005). Rapamycin and UCN-01 synergistically induce apoptosis in human leukemia cells through a process that is regulated by the Raf-1/MEK/ERK, Akt, and JNK signal transduction pathways. *Molecular Cancer Therapeutics*, Vol.4, No.3, pp. 457-470, ISSN 1535-7163

Hashimoto, K.,I. Matsumura, et al. (2003). Necessity of tyrosine 719 and phosphatidylinositol 3'-kinase-mediated signal pathway in constitutive activation and oncogenic potential of c-kit receptor tyrosine kinase with the Asp814Val mutation. *Blood*, Vol.101, No.3, pp. 1094-1102, ISSN 0006-4971

Hawkins, P. T.,K. E. Anderson, et al. (2006). Signalling through Class I PI3Ks in mammalian cells. *Biochemical Society Transactions*, Vol.34, No.Pt 5, pp. 647-662, ISSN 0300-5127

Helgason, C. D.,J. Antonchuk, et al. (2003). Homeostasis and regeneration of the hematopoietic stem cell pool are altered in SHIP-deficient mice. *Blood*, Vol.102, No.10, pp. 3541-3547, ISSN 0006-4971

Helgason, C. D.,J. E. Damen, et al. (1998). Targeted disruption of SHIP leads to hemopoietic perturbations, lung pathology, and a shortened life span. *Genes and Development*, Vol.12, No.11, pp. 1610-1620, ISSN 0890-9369

Hirano, I.,S. Nakamura, et al. (2009). Depletion of Pleckstrin homology domain leucine-rich repeat protein phosphatases 1 and 2 by Bcr-Abl promotes chronic myelogenous leukemia cell proliferation through continuous phosphorylation of Akt isoforms. *Journal of Biological Chemistry*, Vol.284, No.33, pp. 22155-22165, ISSN 0021-9258

Horn, S.,U. Bergholz, et al. (2008). Mutations in the catalytic subunit of class IA PI3K confer leukemogenic potential to hematopoietic cells. *Oncogene*, Vol.27, No.29, pp. 4096-4106, ISSN 1476-5594

Horton, T. M.,D. Pati, et al. (2007). A phase 1 study of the proteasome inhibitor bortezomib in pediatric patients with refractory leukemia: a Children's Oncology Group study. *Clinical Cancer Research*, Vol.13, No.5, pp. 1516-1522, ISSN 1078-0432

Hsieh, A. C.,M. Costa, et al. (2010). Genetic dissection of the oncogenic mTOR pathway reveals druggable addiction to translational control via 4EBP-eIF4E. *Cancer Cell*, Vol.17, No.3, pp. 249-261, ISSN 1878-3686

Huang, J.,Y. Zhang, et al. (2009). Pivotal role for glycogen synthase kinase-3 in hematopoietic stem cell homeostasis in mice. *The Journal of Clinical Investigation*, Vol.119, No.12, pp. 3519-3529, ISSN 1558-8238

Inoki, K.,Y. Li, et al. (2002). TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. *Nature Cell Biology*, Vol.4, No.9, pp. 648-657, ISSN 1465-7392

Inoki, K.,H. Ouyang, et al. (2006). TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth. *Cell*, Vol.126, No.5, pp. 955-968, ISSN 0092-8674

Janes, M. R.,J. J. Limon, et al. (2010). Effective and selective targeting of leukemia cells using a TORC1/2 kinase inhibitor. *Nature Medicine*, Vol.16, No.2, pp. 205-213, ISSN 1546-170X

Jia, Y.,F. Loison, et al. (2008). Inositol trisphosphate 3-kinase B (InsP3KB) as a physiological modulator of myelopoiesis. *Proceedings of the National Academy of Sciences of the United States of America*, Vol.105, No.12, pp. 4739-4744, ISSN 1091-6490

Jia, Y.,K. K. Subramanian, et al. (2007). Inositol 1,3,4,5-tetrakisphosphate negatively regulates phosphatidylinositol-3,4,5- trisphosphate signaling in neutrophils. *Immunity*, Vol.27, No.3, pp. 453-467, ISSN 1074-7613

Jotta, P. Y.,M. A. Ganazza, et al. (2010). Negative prognostic impact of PTEN mutation in pediatric T-cell acute lymphoblastic leukemia. *Leukemia*, Vol.24, No.1, pp. 239-242, ISSN 1476-5551

Jou, S. T.,N. Carpino, et al. (2002). Essential, nonredundant role for the phosphoinositide 3-kinase p110delta in signaling by the B-cell receptor complex. *Molecular and Cellular Biology*, Vol.22, No.24, pp. 8580-8591, ISSN 0270-7306

Juntilla, M. M.,V. D. Patil, et al. (2010). AKT1 and AKT2 maintain hematopoietic stem cell function by regulating reactive oxygen species. *Blood*, Vol.115, No.20, pp. 4030-4038, ISSN 1528-0020

Kharas, M. G.,M. R. Janes, et al. (2008). Ablation of PI3K blocks BCR-ABL leukemogenesis in mice, and a dual PI3K/mTOR inhibitor prevents expansion of human BCR-ABL+ leukemia cells. *The Journal of Clinical Investigation*, Vol.118, No.9, pp. 3038-3050, ISSN 0021-9738

Kharas, M. G.,R. Okabe, et al. (2010). Constitutively active AKT depletes hematopoietic stem cells and induces leukemia in mice. *Blood*, Vol.115, No.7, pp. 1406-1415, ISSN 1528-0020

Kojima, K.,M. Shimanuki, et al. (2008). The dual PI3 kinase/mTOR inhibitor PI-103 prevents p53 induction by Mdm2 inhibition but enhances p53-mediated mitochondrial apoptosis in p53 wild-type AML. *Leukemia*, Vol.22, No.9, pp. 1728-1736, ISSN 1476-5551

Kondapaka, S. B.,S. S. Singh, et al. (2003). Perifosine, a novel alkylphospholipid, inhibits protein kinase B activation. *Molecular Cancer Therapeutics*, Vol.2, No.11, pp. 1093-1103, ISSN 1535-7163

Kops, G. J.,N. D. de Ruiter, et al. (1999). Direct control of the Forkhead transcription factor AFX by protein kinase B. *Nature*, Vol.398, No.6728, pp. 630-634, ISSN 0028-0836

Kornblau, S. M.,N. Singh, et al. (2010). Highly phosphorylated FOXO3A is an adverse prognostic factor in acute myeloid leukemia. *Clinical Cancer Research*, Vol.16, No.6, pp. 1865-1874, ISSN 1078-0432

Kornblau, S. M.,M. Womble, et al. (2006). Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia. *Blood*, Vol.108, No.7, pp. 2358-2365, ISSN 0006-4971

Kubota, Y.,H. Ohnishi, et al. (2004). Constitutive activation of PI3K is involved in the spontaneous proliferation of primary acute myeloid leukemia cells: direct evidence of PI3K activation. *Leukemia*, Vol.18, No.8, pp. 1438-1440, ISSN 0887-6924

Lee, J. W.,Y. H. Soung, et al. (2005). PIK3CA gene is frequently mutated in breast carcinomas and hepatocellular carcinomas. *Oncogene*, Vol.24, No.8, pp. 1477-1480, ISSN 0950-9232

Legrand, O.,G. Simonin, et al. (1999). Simultaneous activity of MRP1 and Pgp is correlated with in vitro resistance to daunorubicin and with in vivo resistance in adult acute myeloid leukemia. *Blood*, Vol.94, No.3, pp. 1046-1056, ISSN 0006-4971

Levy, D. S.,J. A. Kahana, et al. (2009). AKT inhibitor, GSK690693, induces growth inhibition and apoptosis in acute lymphoblastic leukemia cell lines. *Blood*, Vol.113, No.8, pp. 1723-1729, ISSN 1528-0020

Ligresti, G.,L. Militello, et al. (2009). PIK3CA mutations in human solid tumors: role in sensitivity to various therapeutic approaches. *Cell Cycle*, Vol.8, No.9, pp. 1352-1358, ISSN 1551-4005

Liu, Q.,G. Nozari, et al. (1998). Single-tube polymerase chain reaction for rapid diagnosis of the inversion hotspot of mutation in hemophilia A. *Blood*, Vol.92, No.4, pp. 1458-1459, ISSN 0006-4971

Liu, Q.,T. Sasaki, et al. (1999). SHIP is a negative regulator of growth factor receptor-mediated PKB/Akt activation and myeloid cell survival. *Genes and Development*, Vol.13, No.7, pp. 786-791, ISSN 0890-9369

Liu, T. C.,P. M. Lin, et al. (2000). Mutation analysis of PTEN/MMAC1 in acute myeloid leukemia. *Am J Hematol*, Vol.63, No.4, pp. 170-175, ISSN 0361-8609

Lo, T. C.,L. M. Barnhill, et al. (2009). Inactivation of SHIP1 in T-cell acute lymphoblastic leukemia due to mutation and extensive alternative splicing. *Leukemia Research*, Vol.33, No.11, pp. 1562-1566, ISSN 1873-5835

Luo, J. M.,H. Yoshida, et al. (2003). Possible dominant-negative mutation of the SHIP gene in acute myeloid leukemia. *Leukemia*, Vol.17, No.1, pp. 1-8, ISSN 0887-6924

Maehama, T. and J. E. Dixon. (1998). The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *Journal of Biological Chemistry*, Vol.273, No.22, pp. 13375-13378, ISSN 0021-9258

Mahadevan, D. and A. F. List. (2004). Targeting the multidrug resistance-1 transporter in AML: molecular regulation and therapeutic strategies. *Blood*, Vol.104, No.7, pp. 1940-1951, ISSN 0006-4971

Maira, S. M.,F. Stauffer, et al. (2008). Identification and characterization of NVP-BEZ235, a new orally available dual phosphatidylinositol 3-kinase/mammalian target of rapamycin inhibitor with potent in vivo antitumor activity. *Molecular Cancer Therapy*, Vol.7, No.7, pp. 1851-1863, ISSN 1535-7163

Manning, B. D. and L. C. Cantley. (2007). AKT/PKB signaling: navigating downstream. *Cell*, Vol.129, No.7, pp. 1261-1274, ISSN 0092-8674

Mao, X.,B. Cao, et al. (2011). A small-molecule inhibitor of D-cyclin transactivation displays preclinical efficacy in myeloma and leukemia via phosphoinositide 3-kinase pathway. *Blood*, Vol.117, No.6, pp. 1986-1997, ISSN 1528-0020

Meyer, L. H.,S. M. Eckhoff, et al. (2011). Early Relapse in ALL Is Identified by Time to Leukemia in NOD/SCID Mice and Is Characterized by a Gene Signature Involving Survival Pathways. *Cancer Cell*, Vol.19, No.2, pp. 206-217, ISSN 1878-3686

Min, Y. H.,J. I. Eom, et al. (2003). Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable. *Leukemia*, Vol.17, No.5, pp. 995-997, ISSN 0887-6924

Minucci, S. and P. G. Pelicci. (2006). Histone deacetylase inhibitors and the promise of epigenetic (and more) treatments for cancer. *Nature Reviews Cancer*, Vol.6, No.1, pp. 38-51, ISSN 1474-175X

Miyamoto, K.,K. Y. Araki, et al. (2007). Foxo3a is essential for maintenance of the hematopoietic stem cell pool. *Cell Stem Cell*, Vol.1, No.1, pp. 101-112, ISSN 1875-9777

Miyamoto, K.,T. Miyamoto, et al. (2008). FoxO3a regulates hematopoietic homeostasis through a negative feedback pathway in conditions of stress or aging. *Blood*, Vol.112, No.12, pp. 4485-4493, ISSN 1528-0020

Moody, J. L.,L. Xu, et al. (2004). Anemia, thrombocytopenia, leukocytosis, extramedullary hematopoiesis, and impaired progenitor function in Pten+/-SHIP-/- mice: a novel model of myelodysplasia. *Blood*, Vol.103, No.12, pp. 4503-4510, ISSN 0006-4971

Nave, B. T.,M. Ouwens, et al. (1999). Mammalian target of rapamycin is a direct target for protein kinase B: identification of a convergence point for opposing effects of insulin and amino-acid deficiency on protein translation. *The Biochemical Journal*, Vol.344 Pt 2, pp. 427-431, ISSN 0264-6021

Neri, L. M.,P. Borgatti, et al. (2003). The phosphoinositide 3-kinase/AKT1 pathway involvement in drug and all-trans-retinoic acid resistance of leukemia cells. *Molecular Cancer Research*, Vol.1, No.3, pp. 234-246, ISSN 1541-7786

Nguyen, N. Y.,M. J. Maxwell, et al. (2011). An ENU-induced mouse mutant of SHIP1 reveals a critical role of the stem cell isoform for suppression of macrophage activation. *Blood*, pp. ISSN 1528-0020

Ning, Z. Q.,J. Li, et al. (2001). Signal transducer and activator of transcription 3 activation is required for Asp(816) mutant c-Kit-mediated cytokine-independent survival and proliferation in human leukemia cells. *Blood*, Vol.97, No.11, pp. 3559-3567, ISSN 0006-4971

Nishioka, C.,T. Ikezoe, et al. (2008). Blockade of mTOR signaling potentiates the ability of histone deacetylase inhibitor to induce growth arrest and differentiation of acute myelogenous leukemia cells. *Leukemia*, Vol.22, No.12, pp. 2159-2168, ISSN 1476-5551

Nyakern, M.,P. L. Tazzari, et al. (2006). Frequent elevation of Akt kinase phosphorylation in blood marrow and peripheral blood mononuclear cells from high-risk myelodysplastic syndrome patients. *Leukemia*, Vol.20, No.2, pp. 230-238, ISSN 0887-6924

Okkenhaug, K.,A. Bilancio, et al. (2002). Impaired B and T cell antigen receptor signaling in p110delta PI 3-kinase mutant mice. *Science*, Vol.297, No.5583, pp. 1031-1034, ISSN 1095-9203

Oshiro, N.,K. Yoshino, et al. (2004). Dissociation of raptor from mTOR is a mechanism of rapamycin-induced inhibition of mTOR function. *Genes to Cells*, Vol.9, No.4, pp. 359-366, ISSN 1356-9597

Pabst, T.,B. U. Mueller, et al. (2001). Dominant-negative mutations of CEBPA, encoding CCAAT/enhancer binding protein-alpha (C/EBPalpha), in acute myeloid leukemia. *Nature Genetics*, Vol.27, No.3, pp. 263-270, ISSN 1061-4036

Papa, V.,P. L. Tazzari, et al. (2008). Proapoptotic activity and chemosensitizing effect of the novel Akt inhibitor perifosine in acute myelogenous leukemia cells. *Leukemia*, Vol.22, No.1, pp. 147-160, ISSN 1476-5551

Park, S.,N. Chapuis, et al. (2008). PI-103, a dual inhibitor of Class IA phosphatidylinositide 3-kinase and mTOR, has antileukemic activity in AML. *Leukemia*, Vol.22, No.9, pp. 1698-1706, ISSN 1476-5551

Perez, L. E.,C. Desponts, et al. (2008). SH2-inositol phosphatase 1 negatively influences early megakaryocyte progenitors. *PLoS One*, Vol.3, No.10, pp. e3565, ISSN 1932-6203

Pouillon, V.,R. Hascakova-Bartova, et al. (2003). Inositol 1,3,4,5-tetrakisphosphate is essential for T lymphocyte development. *Nature Immunology*, Vol.4, No.11, pp. 1136-1143, ISSN 1529-2908

Rahmani, M.,E. Reese, et al. (2005). Coadministration of histone deacetylase inhibitors and perifosine synergistically induces apoptosis in human leukemia cells through Akt and ERK1/2 inactivation and the generation of ceramide and reactive oxygen species. *Cancer Research*, Vol.65, No.6, pp. 2422-2432, ISSN 0008-5472

Raslova, H.,V. Baccini, et al. (2006). Mammalian target of rapamycin (mTOR) regulates both proliferation of megakaryocyte progenitors and late stages of megakaryocyte differentiation. *Blood*, Vol.107, No.6, pp. 2303-2310, ISSN 0006-4971

Raynaud, F. I.,S. Eccles, et al. (2007). Pharmacologic characterization of a potent inhibitor of class I phosphatidylinositide 3-kinases. *Cancer Research*, Vol.67, No.12, pp. 5840-5850, ISSN 0008-5472

Recher, C.,O. Beyne-Rauzy, et al. (2005). Antileukemic activity of rapamycin in acute myeloid leukemia. *Blood*, Vol.105, No.6, pp. 2527-2534, ISSN 0006-4971

Rizzieri, D. A.,E. Feldman, et al. (2008). A phase 2 clinical trial of deforolimus (AP23573, MK-8669), a novel mammalian target of rapamycin inhibitor, in patients with relapsed or refractory hematologic malignancies. *Clinical Cancer Research*, Vol.14, No.9, pp. 2756-2762, ISSN 1078-0432

Rodriguez-Viciana, P.,P. H. Warne, et al. (1994). Phosphatidylinositol-3-OH kinase as a direct target of Ras. *Nature*, Vol.370, No.6490, pp. 527-532, ISSN 0028-0836

Sampath, D.,J. Cortes, et al. (2006). Pharmacodynamics of cytarabine alone and in combination with 7-hydroxystaurosporine (UCN-01) in AML blasts in vitro and during a clinical trial. *Blood*, Vol.107, No.6, pp. 2517-2524, ISSN 0006-4971

Santamaria, C. M.,M. C. Chillon, et al. (2009). High FOXO3a expression is associated with a poorer prognosis in AML with normal cytogenetics. *Leukemia Research*, Vol.33, No.12, pp. 1706-1709, ISSN 1873-5835

Sarbassov, D. D.,D. A. Guertin, et al. (2005). Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. *Science*, Vol.307, No.5712, pp. 1098-1101, ISSN 1095-9203

Saunders, P.,A. Cisterne, et al. (2011). The mammalian target of rapamycin inhibitor RAD001 (everolimus) synergizes with chemotherapeutic agents, ionizing radiation and proteasome inhibitors in pre-B acute lymphocytic leukemia. *Haematologica*, Vol.96, No.1, pp. 69-77, ISSN 1592-8721

Schubbert, S.,K. Shannon, et al. (2007). Hyperactive Ras in developmental disorders and cancer. *Nature Reviews Cancer*, Vol.7, No.4, pp. 295-308, ISSN 1474-175X

Silva, A.,J. A. Yunes, et al. (2008). PTEN posttranslational inactivation and hyperactivation of the PI3K/Akt pathway sustain primary T cell leukemia viability. *The Journal of Clinical Investigation*, Vol.118, No.11, pp. 3762-3774, ISSN 0021-9738

Skorski, T.,A. Bellacosa, et al. (1997). Transformation of hematopoietic cells by BCR/ABL requires activation of a PI-3k/Akt-dependent pathway. *EMBO Journal*, Vol.16, No.20, pp. 6151-6161, ISSN 0261-4189

Sujobert, P.,V. Bardet, et al. (2005). Essential role for the p110delta isoform in phosphoinositide 3-kinase activation and cell proliferation in acute myeloid leukemia. *Blood*, Vol.106, No.3, pp. 1063-1066, ISSN 0006-4971

Tabellini, G.,P. L. Tazzari, et al. (2004). Novel 2'-substituted, 3'-deoxy-phosphatidyl-myo-inositol analogues reduce drug resistance in human leukaemia cell lines with an activated phosphoinositide 3-kinase/Akt pathway. *British Journal of Haematology*, Vol.126, No.4, pp. 574-582, ISSN 0007-1048

Tamburini, J.,N. Chapuis, et al. (2008). Mammalian target of rapamycin (mTOR) inhibition activates phosphatidylinositol 3-kinase/Akt by up-regulating insulin-like growth factor-1 receptor signaling in acute myeloid leukemia: rationale for therapeutic inhibition of both pathways. *Blood*, Vol.111, No.1, pp. 379-382, ISSN 0006-4971

Tamburini, J.,C. Elie, et al. (2007). Constitutive phosphoinositide 3-kinase/Akt activation represents a favorable prognostic factor in de novo acute myelogenous leukemia patients. *Blood*, Vol.110, No.3, pp. 1025-1028, ISSN 0006-4971

Tazzari, P. L.,G. Tabellini, et al. (2008). Synergistic proapoptotic activity of recombinant TRAIL plus the Akt inhibitor Perifosine in acute myelogenous leukemia cells. *Cancer Research*, Vol.68, No.22, pp. 9394-9403, ISSN 1538-7445

Teachey, D. T.,D. A. Obzut, et al. (2006). The mTOR inhibitor CCI-779 induces apoptosis and inhibits growth in preclinical models of primary adult human ALL. *Blood*, Vol.107, No.3, pp. 1149-1155, ISSN 0006-4971

Teachey, D. T.,C. Sheen, et al. (2008). mTOR inhibitors are synergistic with methotrexate: an effective combination to treat acute lymphoblastic leukemia. *Blood*, Vol.112, No.5, pp. 2020-2023, ISSN 1528-0020

Thomas, X. (2009). Chemotherapy of acute leukemia in adults. *Expert Opinion on Pharmacotherapy*, Vol.10, No.2, pp. 221-237, ISSN 1744-7666

Tothova, Z.,R. Kollipara, et al. (2007). FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. *Cell*, Vol.128, No.2, pp. 325-339, ISSN 0092-8674

Trowbridge, J. J.,A. Xenocostas, et al. (2006). Glycogen synthase kinase-3 is an in vivo regulator of hematopoietic stem cell repopulation. *Nature Medicine*, Vol.12, No.1, pp. 89-98, ISSN 1078-8956

van de Laar, L.,M. Buitenhuis, et al. (2010). Human CD34-derived myeloid dendritic cell development requires intact phosphatidylinositol 3-kinase-protein kinase B-mammalian target of rapamycin signaling. *Journal of Immunology*, Vol.184, No.12, pp. 6600-6611, ISSN 1550-6606

Vanhaesebroeck, B.,S. J. Leevers, et al. (2001). Synthesis and function of 3-phosphorylated inositol lipids. *Annu Rev Biochem*, Vol.70, pp. 535-602, ISSN 0066-4154

Varticovski, L.,G. Q. Daley, et al. (1991). Activation of phosphatidylinositol 3-kinase in cells expressing abl oncogene variants. *Molecular and Cellular Biology*, Vol.11, No.2, pp. 1107-1113, ISSN 0270-7306

Vlahos, C. J.,W. F. Matter, et al. (1994). A specific inhibitor of phosphatidylinositol 3-kinase, 2-(4-morpholinyl)-8-phenyl-4H-1-benzopyran-4-one (LY294002). *Journal of Biological Chemistry*, Vol.269, No.7, pp. 5241-5248, ISSN 0021-9258

Wang, C.,X. Chen, et al. (2007). C/EBPalphap30 plays transcriptional regulatory roles distinct from C/EBPalphap42. *Cell Res*, Vol.17, No.4, pp. 374-383, ISSN 1748-7838

Weisberg, E.,L. Banerji, et al. (2008). Potentiation of antileukemic therapies by the dual PI3K/PDK-1 inhibitor, BAG956: effects on BCR-ABL- and mutant FLT3-expressing cells. *Blood*, Vol.111, No.7, pp. 3723-3734, ISSN 0006-4971

Wymann, M. P.,G. Bulgarelli-Leva, et al. (1996). Wortmannin inactivates phosphoinositide 3-kinase by covalent modification of Lys-802, a residue involved in the phosphate transfer reaction. *Molecular and Cellular Biology*, Vol.16, No.4, pp. 1722-1733, ISSN 0270-7306

Xu, Q.,S. E. Simpson, et al. (2003). Survival of acute myeloid leukemia cells requires PI3 kinase activation. *Blood*, Vol.102, No.3, pp. 972-980, ISSN 0006-4971

Xu, Q.,J. E. Thompson, et al. (2005). mTOR regulates cell survival after etoposide treatment in primary AML cells. *Blood*, Vol.106, No.13, pp. 4261-4268, ISSN 0006-4971

Yap, T. A.,M. D. Garrett, et al. (2008). Targeting the PI3K-AKT-mTOR pathway: progress, pitfalls, and promises. *Current Opinion in Pharmacology*, Vol.8, No.4, pp. 393-412, ISSN 1471-4892

Yilmaz, O. H.,R. Valdez, et al. (2006). Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. *Nature*, Vol.441, No.7092, pp. 475-482, ISSN 1476-4687

Yoshimi, A.,S. Goyama, et al. (2011). Evi1 represses PTEN expression by interacting with polycomb complexes and activates PI3K/AKT/mTOR signaling. *Blood*, pp. ISSN 1528-0020

Yuan, R.,A. Kay, et al. (2009). Targeting tumorigenesis: development and use of mTOR inhibitors in cancer therapy. *Journal of Hematology and Oncology*, Vol.2, pp. 45, ISSN 1756-8722

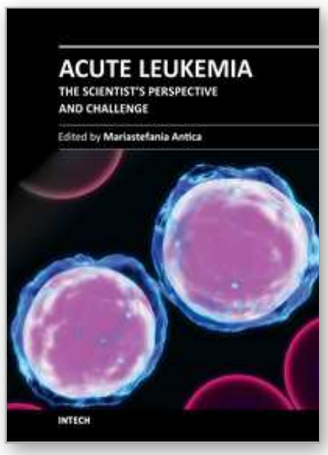
Zask, A.,J. Kaplan, et al. (2008). Synthesis and structure-activity relationships of ring-opened 17-hydroxywortmannins: potent phosphoinositide 3-kinase inhibitors with improved properties and anticancer efficacy. *Journal of Medicinal Chemistry*, Vol.51, No.5, pp. 1319-1323, ISSN 0022-2623

Zeng, Z.,I. J. Samudio, et al. (2006). Simultaneous inhibition of PDK1/AKT and Fms-like tyrosine kinase 3 signaling by a small-molecule KP372-1 induces mitochondrial dysfunction and apoptosis in acute myelogenous leukemia. *Cancer Research*, Vol.66, No.7, pp. 3737-3746, ISSN 0008-5472

Zhang, J.,J. C. Grindley, et al. (2006). PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. *Nature*, Vol.441, No.7092, pp. 518-522, ISSN 1476-4687

Zhao, S.,M. Konopleva, et al. (2004). Inhibition of phosphatidylinositol 3-kinase dephosphorylates BAD and promotes apoptosis in myeloid leukemias. *Leukemia*, Vol.18, No.2, pp. 267-275, ISSN 0887-6924

Zhao, W. L. (2010). Targeted therapy in T-cell malignancies: dysregulation of the cellular signaling pathways. *Leukemia*, Vol.24, No.1, pp. 13-21, ISSN 1476-5551



Acute Leukemia - The Scientist's Perspective and Challenge
Edited by Prof. Mariastefania Antica

ISBN 978-953-307-553-2
Hard cover, 428 pages
Publisher InTech
Published online 22, December, 2011
Published in print edition December, 2011

This book provides a comprehensive overview of he basic mechanisms underlying areas of acute leukemia, current advances, and future directions in management of this disease. The first section discusses the classification of acute leukemia, taking into account diagnoses dependent on techniques that are essential, and thankfully readily available, in the laboratory. The second section concerns recent advances in molecular biology, markers, receptors, and signaling molecules responsible for disease progression, diagnostics based on biochips and other molecular genetic analysis. These advances provide clinicians with important understanding and improved decision making towards the most suitable therapy for acute leukemia. Biochemical, structural, and genetic studies may bring a new era of epigenetic based drugs along with additional molecular targets that will form the basis for novel treatment strategies. Later in the book, pediatric acute leukemia is covered, emphasizing that children are not small adults when it comes to drug development. The last section is a collection of chapters about treatment, as chemotherapy-induced toxicity is still a significant clinical concern. The present challenge lies in reducing the frequency and seriousness of adverse effects while maintaining efficacy and avoiding over-treatment of patients.

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Roel Polak and Miranda Buitenhuis (2011). The PI3K/PKB Signaling Module in Normal and Malignant Hematopoiesis, Acute Leukemia - The Scientist's Perspective and Challenge, Prof. Mariastefania Antica (Ed.), ISBN: 978-953-307-553-2, InTech, Available from: http://www.intechopen.com/books/acute-leukemia-the-scientist-s-perspective-and-challenge/the-pi3k-pkb-signaling-module-in-normal-and-malignant-hematopoiesis

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen