haematologica
the hematology journal

Early Release Paper

Role of the PI3K/AKT and mTOR signalling pathways in acute myeloid leukemia

by Sophie Park, Nicolas Chapuis, Jerome Tamburini, Valerie Bardet, Pascale Cornillet-Lefebvre, Lise Willems, Alexa Green, Patrick Mayeux, Catherine Lacombe, and Didier Bouscary

Haematologica 2009 [Epub ahead of print]

doi:10.3324/haematol.2009.013797

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature. Haematologica is the official organ of the European Hematology Association (www.ehaweb.org).

DOI: 10.3324/haematol.2009.013797

Role of the PI3K/AKT and mTOR signalling pathways in acute myeloid leukemia

Sophie Park [1,2,3,5,7], Nicolas Chapuis [1,2,3,4], Jérôme Tamburini [1,2,3,5,7], Valérie Bardet [1,2,3,4], Pascale Cornillet-Lefebvre [6,7], Lise Willems [1,2,3], Alexa Green [1,2,3], Patrick Mayeux [1,2,3], Catherine Lacombe [1,2,3,4,7], Didier Bouscary [1,2,3,5,7]

From : [1]Institut Cochin, Département d'Hématologie, CNRS, UMR8104, Paris, France; [2]INSERM, U567, Paris, France; [3]Université Paris Descartes, Faculté de Médecine René Descartes, Paris, France; [4]Service d'Hématologie Biologique, Hôpital Cochin, AP-HP, Paris, France; [5]Service de Médecine Interne - UF d'Hématologie, Hôpital Cochin, AP-HP, Paris, France; [6]Laboratoire d'hématologie, Centre Hospitalo-Universitaire (CHU) Reims, France; [7]Groupe Ouest Est des Leucémies et Autres Maladies du Sang (GOELAMS), France.

Correspondence

Didier Bouscary, Département d'Immunologie-Hématologie,

Equipe 35, Hôpital. Cochin, 27, rue du Faubourg Saint-Jacques F-75014 Paris, France

E-mail : didier.bouscary@inserm.fr; didier.bouscary@cch.aphp.fr

Tel: (33) 1 58 41 21 00; Fax: (33) 1 58 41 20 80

DOI: 10.3324/haematol.2009.013797

Abstract

The PI3K/AKT and mTOR signalling pathways are activated in acute myeloid leukemia (AML), including in the more immature leukemic populations. Constitutive PI3K activation is detectable in 50% of AML samples whereas mTORC1 is activated in all cases of this disease. In leukemic cells, the PI3K activity relates to the expression of the p110δ isoform of class IA PI3K. Constitutive PI3K activation is the result of autocrine IGF-1/IGF-1R signalling in 70% of AML samples but specific inhibition of this pathway does not induce apoptosis. Specific inhibition of PI3K/AKT or mTORC1 alone *in vitro* has anti-leukemic effects which are essentially exerted via the suppression of proliferation. However, as mTORC1 activation is independent of PI3K/AKT in AML, dual PI3K and mTOR inhibitors may induce apoptosis in blast cells. Moreover, mTORC1 inhibition using sirolimus overactivates PI3K/AKT via the up-regulation of IRS2 expression and by favouring IGF-1/IGF-1R autocrine signalling. Recent data also indicate that mTORC1 does not control protein translation in AML. These results open avenues for the design of direct inhibitors of protein synthesis as novel AML therapies and also for the development of second generation mTOR inhibitors (the TORKinhibs).

DOI: 10.3324/haematol.2009.013797

1/ Introduction

Acute myeloid leukemia (AML) comprises a group of clonal malignant diseases characterised by a deregulated proliferation of immature myeloid cells (1). Most AML patients who receive intensive chemotherapy achieve complete remission but the frequency of relapse is high and the overall five year survival rate is only 20% (2). AML is characterized by the uncontrolled proliferation/survival of immature myeloid progenitors that undergo a differentiation block at various maturation steps, leading to the accumulation of leukemic cells in the bone marrow and inhibition of normal haematopoiesis (3). Leukemic haematopoiesis shares similarities with normal haematopoiesis (4, 5) and the oncogenic events associated with these cancers may arise either directly in a hematopoietic stem cell, or in a myeloid progenitor devoid of intrinsic self-renewal potential (4-6). In AML, deregulation of the signalling pathways that enhance the survival and proliferation of hematopoietic progenitor cells cooperates with abnormalities in the functions of transcription factors implicated in normal myeloid differentiation to induce leukemia (7). In this regard, the abnormal activation of PI3K/AKT, mTORC1, ERK/MAPK, STAT3/5, Wnt/β-catenin, and NF-κB has been reported (8-20). It has been postulated that the effective targeting of some of these pathways could have a major impact on AML treatment. This review focuses on the class IA PI3K/AKT and mTOR signalling pathways and on recent data concerning their role, mechanisms of activation and interactions in AML biology.

2/ General biology of the class IA PI3K and mTOR signalling pathways

There are three classes of PI3K (I-III) each with its own substrate specificity and lipid products (21, 22). The following section describes the general biology of the PI3K/AKT pathway, focusing on class IA PI3K which has the strongest associations with cancer (23, 24). Class IA PI3Ks are heterodimers composed of a p110 catalytic subunit (α [PK3CA], β [PK3CB] or δ [PK3CD]) and a p50/p55/p85 regulatory subunit and are activated via

DOI: 10.3324/haematol.2009.013797

tyrosine kinase receptors (TKR). Activated PI3K phosphorylates the lipid phosphatidyl-inositol bisphosphate (PIP2) to generate phosphatidyl-inositol trisphosphate (PIP3) and thereby initiate the activation of the Ser/Thr kinase AKT. PIP3 recruits PDK1 and AKT to the plasma membrane, where PDK1 phosphorylates AKT on Thr308 in the activation loop of the kinase domain. The phosphorylation of AKT on Ser473 by PDK2 acts as a "gain control" for AKT and regulates its degree of activation (Figure 1). The sirolimus-insensitive mTORC2 complex exhibits PDK2 activity and is described below (Figure 2).

The AKT network controls different targets including the FOXO family of transcription factors. When they are unphosphorylated, the FOXOs (FOXO1, FOXO3A, FOXO4) localize in the nucleus and induce the transcription of a wide array of target genes involved in the cell cycle and apoptosis such as CDN1B ($p27^{Kip1}$) and CDN1A ($p21^{Cip1}$), Fas-L (TNFL6) and BIM (25). PI3K activation downstream from growth factor receptors (26) negatively regulates FOXO proteins (Figure 1). AKT phosphorylates FOXO3a at three conserved sites (Thr32, Ser253 and Ser315), therefore creating binding sites for the 14-3-3 chaperone proteins and leading to the active export of FOXO3a to the cytoplasm where it is targeted for proteasomal degradation (25).

The PI3K activity that results in PIP3 production is tightly controlled and negatively regulated by several phosphatases. The PTEN lipid phosphatase dephosphorylates PIP3 at the 3' position, whereas SHIP-1 phosphatase dephosphorylates it at the 5' position, in both cases limiting the production of PIP3 (27). Genetic inactivation of PTEN leads to constitutive activation of the PI3K/AKT/mTORC1 axis (28). Phosphorylation of AKT on Ser473 and Thr308 is also negatively regulated by the PH domain leucine-rich repeat Protein Phosphatase (PHLPP) and by PP2A, respectively (29) (Figure 2). Moreover, it has been shown that the PML tumour suppressor inactivates phosphorylated AKT in the nucleus i.e. PML specifically recruits AKT and its phosphatase PP2A to the nuclear bodies (30).

MTORC1 activation is regulated by different upstream mechanisms, mostly convergent on the TSC1/TSC2/Rheb axis. These include AKT, which, by phosphorylating TSC2 disrupts the GAP activity of the TSC2/TSC1 complex for RHEB, a RAS homologue

DOI: 10.3324/haematol.2009.013797

(31), thereby allowing RHEB-GTP to activate mTORC1. The mTORC1 complex is composed of FRAP (mTOR), Raptor, PRAS40 and LSt8 (GβL)(32) and regulates protein synthesis by activating p70S6K and inactivating 4EBP1. p70S6K is thought to positively regulate protein synthesis and cell size by controlling the translation of mRNA with 5'-terminal oligopyrimidine tracts (33, 34). Unphosphorylated 4EBP1 interacts with the cap-binding protein eIF4E and prevents the formation of the 4F translational initiation complex (eIF4F), by competing for the fixation of eIF4G to eIF4E. When phosphorylated by mTORC1, 4EBP1 dissociates from eIF4E and initiates the formation of the eIF4F complex which then translates the oncogenic cap-mRNAs, including MYC, HIF-1, CCND1 (cyclin-D1) and Bcl-XL (35). MTORC1 activity is specifically inhibited by sirolimus (rapamycin, Wyeth), a drug that interacts with the FKB1A (FKBP12) protein and binds the FRAP domain of the mTOR kinase.

The interactions between mTORC1 and PI3K are complex. MTORC1 activity negatively regulates PI3K (36). In normal cells with mTORC1 activity, and in cells with constitutive mTORC1 activity due to germ line mutations in the TSC1/TSC2 complex, S6K activation downstream of mTORC1 phosphorylates the IRS proteins on Ser residues, targeting them for proteasomal degradation (36) (Figure 2). Inhibition of the mTORC1 pathway by sirolimus removes this negative feed-back loop and activates AKT. Other data suggest that IRS-2 can upregulate the expression of the IGF-1R at the protein level and also that mTORC1 plays a crucial role in this control (37). MTOR is a component of two different complexes in mammalian cells: the mTORC1 complex already described and the mTORC2 complex composed of the FRAP, Protor, LST8, SIN1 and Rictor proteins (Figure 2). MTORC2 has PDK2 activity and phosphorylates AKT on Ser473 (38). MTORC2 also phosphorylates the serum and glucocorticoid protein kinase (SGK) and some isoforms of PKC (39),(40). However, the upstream mechanisms regulating mTORC2 activation are largely unknown. MTORC2 is sirolimus-insensitive but a long-term exposure to sirolimus can disrupt its formation and inhibit AKT Ser473 phosphorylation in some models (41). Recently a new protein known as DEPTOR has been identified as an mTOR-interacting protein (42). Taken together, these data indicate that the connections between both of the mTOR

DOI: 10.3324/haematol.2009.013797

complexes and the AKT pathways are not straightforward i.e. AKT functions upstream of mTORC1 but downstream of mTORC2.

3/ Role of the PI3K and mTORC1 signalling pathways in normal and malignant haematopoiesis

The PI3K/AKT and mTORC1 pathways have been reported to regulate both normal and malignant haematopoiesis (43, 44). In particular, PI3K activity controls erythropoiesis and mediates the proliferation and survival of erythroid progenitors (45, 46). The available evidence also now indicates that AKT regulates lineage fates during myelopoiesis, which is mediated at least in part by controlling the phosphorylation of the transcription factor CEBPA (C/EBPα). Moreover, the inhibition of AKT results in GSK-3 activation, in the phosphorylation and inactivation of CEBPA, and in eosinophil differentiation (47), whereas AKT activation activates CEBPA and enhances neutrophil development (47).

PTEN phosphatase is particularly important in the hematopoietic stem cell (HSC) compartment where it dephosphorylates PIP3 at the 3' position, inhibiting the production of active PIP3 and leading to inhibition of PI3K/AKT signalling. PTEN inactivation in the mouse leads to different outcomes in normal HSCs compared with leukemic stem cells (LSCs). PTEN-deficient HSCs proliferate and have reduced retention in the bone marrow (BM). They also engraft normally in irradiated recipient mice but do not sustain long-term haematopoiesis. PTEN-deficient mice also develop myeloproliferative disorders and sometimes leukemias, most likely as a result of genomic instability. The mechanisms underpinning the different roles of PTEN in normal and LSCs remain unclear however (6, 43, 44). Leukemogenesis in PTEN-mutated mice may be controlled by activated mTORC1 as rapamycin treatment of PTEN deficient mice depletes leukemia-initiating cells and restores normal HSC function (43). Recent data also demonstrate that a loss of TSC1 expression upstream of mTORC1 in HSCs impairs haematopoiesis, drives HSCs from quiescence into

DOI: 10.3324/haematol.2009.013797

rapid cycling, and thereby contributes to a defective long-term repopulating potential (48, 49). However, a TSC1 deletion in HSCs does not induce acute leukemia.

Mice that conditionally lack the FOXO1, FOXO3A and FOXO4 transcription factors in HSCs demonstrate that FOXOs are critical for their long-term maintenance. In this regard, FOXO-deficient BM manifests a defective long-term repopulating activity that correlates with increased cell cycling and apoptosis (50), in tandem with the production of reactive oxygen species (ROS). *In vivo* treatment with the anti-oxidative agent N-acetyl-L-cysteine results in reversion of the FOXO-deficient HSC phenotype. Hence, FOXOs play very important roles in the response to physiologic oxidative stress and mediate quiescence and enhanced survival in the HSC compartment (50).

4/ The role of the PI3K/AKT and mTORC1 signalling pathways in AML

4-1/ Incidence of PI3K/AKT and mTORC1 activation in AML

The PI3K/AKT pathway is frequently activated in AML (10, 51). AKT phosphorylation on Ser473, assayed by western blotting (WB) analysis of purified blast cells, can be detected in 50-80% of AML patients (51-53). Constitutive activation of PI3K/AKT (PI3K+ AML samples) is found in 50% of *de novo* AML samples analyzed after serum and cytokine starvation (10). Less WB data are available concerning AKT Thr308 phosphorylation (54). For hypoblastic patients, flow cytometry analysis of AKT phosphorylation may offer several advantages over WB, including the lower number of blast cells required, the capacity to identify rare immature leukemic populations containing LSCs, and the rapidity of the results (55). This technique can also be used to assess the efficacy of different drugs targeting the PI3K/AKT pathway *ex vivo*. AKT Ser473 phosphorylation can be detected by flow cytometry analysis of the gated $CD45^{low}$ blast cell population and the results correlate with WB data (55). AKT Ser473 phosphorylation is also detectable in the $CD34^{+}$, $CD38^{Low/neg}$, and $CD123^{+}$ population containing immature leukemic cells, but only when AKT is activated in the more

DOI: 10.3324/haematol.2009.013797

differentiated blast cell population, suggesting that PI3K is activated at all stages of leukemic haematopoiesis (55).

The mTORC1 pathway is also activated in AML (56-59). In our hands, constitutive mTORC1 activation, as assessed by phosphorylation of P70S6K on Thr389 by WB, was found to be detectable in almost all of the samples tested (59). The other target of mTORC1, the 4E-BP1 protein, is phosphorylated on Thr70 or on Ser65 in all primary AML samples (58, 59).

4-2/ Mechanisms of PI3K/AKT and mTORC1 activation in AML

The mechanisms leading to PI3K/AKT activation in AML are not completely clear. PK3CD, the p110δ isoform of class IA PI3K, is always expressed in AML cells, whereas the p110α and p110β isoforms are heterogeneously expressed (9, 60). IC87114, a specific PK3CD inhibitor, fully inhibits AKT phosphorylation on Ser473, and also FOXO3 phosphorylation on Thr32, in primary AML cells of all FAB subtypes except AML3 (9, 60). However, in blast cells stimulated with IGF-1, over-increased PI3K activity is also mediated by the p110β isoform recruited to the IGF-1 receptor after stimulation (61). In acute promyelocytic leukemia (APL), class IA PI3K signalling is mediated by both the p110β and p110δ isoforms (62). Inhibition of these two isoforms using specific p110 inhibitors in primary APL cells induces apoptosis in the presence or absence of all-trans-retinoic-acid (62). It is known also that constitutive over-expression of wild type PK3CD is sufficient to activate AKT and to transform cultured cells (63). p110δ is expressed at high level in lymphocytes and myeloid cells (64) and its expression is mainly regulated at the transcriptional level. A highly conserved transcription factor binding cluster was recently identified in the PI3KCD gene with high promoter activity specifically detected in leukocytes (64). This may explain why PI3KCD is always expressed in AML. However, PK3CD expression detected by WB has been found in our laboratory to be identical in PI3K+ and PI3K- AML samples and at similar levels to those found in normal CD34+ cells (our unpublished data). This suggests that the over-

DOI: 10.3324/haematol.2009.013797

expression of PK3CD is not responsible for PI3K/AKT activation. Activating mutations in the PI3KCA gene coding for the p110α isoform have also been detected in different cancers (63). However, no such mutation has been identified in primary AML blast cells (65). Moreover, no activating mutation in the PI3KCD gene encoding p110δ has yet been identified in a large cohort of PI3K+ AML samples (66). The constitutive activation of AKT1 has been reported in cancers and results from a mutation in its PH domain (67). The transforming activity of this E17K-AKT1 mutant is due to a PIP2 and PIP3-independent recruitment of AKT1 to the membrane. This mutation activates AKT1 by means of a pathological localization to the plasma membrane, and thereby stimulates downstream signalling pathways and induces leukemia in mice (68). No E17K PH mutation has yet been identified in AML (54, 69).

Alterations in the activity of the PTEN and SHIP phosphatases that negatively regulate PIP3 production may also activate PI3K in AML but PTEN and SHIP1 mutations are rare in this disease (70). Phosphorylation and the decreased expression of PTEN have been reported, but their significance remains controversial (71). Lentiviral expression of the INPP5D gene encoding SHIP1 in CD34+ cells from AML patients profoundly reduces their GM-CSF-induced proliferation (72). However, more studies are required to determine the exact role of these two phosphatases in AML.

PI3K activity can be induced downstream of activated tyrosine kinase receptors (TKRs). In most PI3K+ samples, constitutive phosphorylation of the GAB1/GAB2 adaptors is detectable, suggesting that a kinase upstream of PI3K is deregulated (our unpublished data). However, no statistical association between the FLT3-ITD and c-Kit mutations has been detected in primary AML cells (10, 66). However, it has been shown that GAB2, which is overexpressed in AML, can mediate PI3K activation downstream of some mutant c-Kit receptors (73, 74).

The IGF-1/IGF-1R signalling pathway also activates PI3K in AML. Leukemic cells express functional IGF-1R and IGF-1 autocrine production is detected in AML blast cells (61, 75, 76). Down-regulation of the PK3CB (p110β)and PK3CD isoforms of PI3K by RNA

DOI: 10.3324/haematol.2009.013797

interference impairs IGF-1-stimulated AKT activation, cell growth and survival in AML cells (61). Using neutralizing anti IGF-1R antibodies (Ab), anti IGF-1 Ab or IGF-1 siRNA in primary blast cells, we have found that constitutive PI3K activation is due to an IGF-1/IGF-1R autocrine loop in 70% of cases (Chapuis et al, Haematologica 2009, in press). Moreover, we report in this same study that the inhibition of AKT phosphorylation with neutralizing anti IGF-1R antibodies blocks the proliferation of blast cells and inhibits the clonogenicity of leukemic progenitors but does not induce significant apoptosis (Chapuis et al, Haematologica 2009, in press). Other potential mechanisms of PI3K activation in AML might also implicate autocrine/paracrine VEGF or angiopoietin secretion (77, 78). Blast cells also directly interact with the bone marrow micro-environment, and ITA4 (VLA-4) / FINC (fibronectin) interactions activate PI3K in blast cells (79).

Given its reported PDK2 activity, the mTORC2 complex is likely to be activated in primary AML cells and might control AKT Ser473 phosphorylation. Long-term treatment with sirolimus or its derivatives (rapalogs) can disrupt the formation of the mTORC2 complex resulting in decreased Ser473 AKT phosphorylation in some cell types, including leukemic cells (80). However, the frequency of this effect in primary AML cells is unknown. In our experience, 24-hour everolimus (Certican, Novartis) exposure inhibits AKT Ser473 phosphorylation in only 1 out of 7 tested samples (our unpublished data). However, the oncogenic role of the mTORC2 complex in AML biology must be specifically addressed. In addition, other kinases can phosphorylate AKT on Ser 473 in AML, such as the integrin linked kinase (ILK) which interacts with beta integrins to phosphorylate AKT. Moreover, bone marrow-derived mesenchymal cells induce ILK and AKT activation in leukemic cells and QLT0267, an ILK inhibitor, reduces stroma-induced AKT phosphorylation. These data strongly suggest that ILK inhibitors may target leukemic cells in their micro-environment (81).The mechanisms potentially contributing to PI3K/AKT activation in AML are listed in Table 1.

The mechanisms leading to constitutive mTORC1 activation in primary AML blast cells are currently unknown. Several studies have indicated that constitutive mTORC1

DOI: 10.3324/haematol.2009.013797

activation, as detected by the phosphorylation of P70S6K on Thr389, is PI3K-independent as a/ in around 50% of AML samples, mTORC1 is activated whereas PI3K/AKT is not (59); b/ IC87114 (ICOS)-induced PI3K/AKT specific inhibition does not suppress mTORC1 activity (76); c/ the Src kinase Lyn is constitutively phosphorylated in some leukemic cells and controls mTORC1 but not AKT activation (82); d/ Inhibition of the IGF-1/IGF-1R autocrine loop with anti-IGF-1R antibodies inhibits AKT phosphorylation but not P70S6K phosphorylation; and e/ mTORC1 activity in AML may be up-regulated through ERK/MAPK (83). Other unexplored signalling pathways may positively or negatively control mTORC1 activity in AML, including the amino acid pathway and the energetic LKB1/AMPK pathway.

4-3/ Functional role of PI3K/AKT and mTORC1 activation in AML

The PI3K/AKT pathway controls blast cell proliferation and the clonogenicity of leukemic progenitors (9, 51) and PI3K inhibition with LY294002 induces apoptosis in these progenitors (9). However, LY294002 inhibits all PI3Ks and also other kinases of the PI3K-related kinase family (PIKK). In contrast, specific inhibition of PK3CD with IC87114 induces low rates of apoptosis, indicating that LY294002-induced apoptosis is related to the inhibition of other targets, such as the mTORC2 complex or the PIM kinases (60). The reasons why the specific inhibition of PI3K does not induce significant apoptosis in primary AML cells are unknown, whereas it is known that PI3K positively controls the phosphorylation of Bad and FOXO3A, two targets downstream of AKT that control cell survival (84).

Rapalogs which belong to the first generation of allosteric mTORC1 inhibitors reduce blast cell proliferation and decrease the clonogenicity of leukemic progenitor cells (56-59) . Surprisingly, these compounds are mainly cytostatic, as long-term treatment induces only very low rates of apoptosis (56-59). However, inhibition of mTORC1 with everolimus has synergistic effects with cytosine arabinoside and etoposide (58). Nevertheless, resistance to sirolimus and its derivatives in AML may be explained by the upregulation of PI3K activity observed after mTORC1 inhibition. Indeed, mTORC1 mediates a negative feedback loop on PI3K, and everolimus-induced mTORC1 inhibition up-regulates AKT S473 phosphorylation

DOI: 10.3324/haematol.2009.013797

by increasing IRS2 expression in primary blast cells, thus promoting an autocrine IGF-1/IGF1R activation loop (76). These data provide a rationale for simultaneously inhibiting both PI3K and mTORC1 (76). This was further supported by the demonstration that PI-103 (85), the first dual PI3K and mTOR inhibitor to become available, has potent anti-leukemic activity and induces significant apoptosis in blast cells and also in immature leukemic cells, with synergistic effects between PI-103 and etoposide to induce blast cell apoptosis (86, 87).

We recently reported that protein synthesis is rapamycin-resistant in primary AML cells (59). Rapamycin or mTORC1 specific disruption with Raptor siRNA inhibits some but not all the functions generally thought to be controlled by mTORC1 in blast cells. MTORC1 inhibition suppresses p70S6K phosphorylation on Thr389 but does not inhibit the 4E-BP1 phosphorylation events that control cap-dependent mRNA translation. Rapamycin-resistant 4E-BP1 phosphorylation is mainly due to PIM2 kinase, which is permanently expressed in primary AML cells (59). Indeed, phosphorylation of Ser65 on 4E-BP1 that is necessary to initiate the formation of the EIF4F initiation complex is controlled by PIM2. Consequently, mTORC1 does not control the synthesis of oncogenic proteins such as c-Myc, Cyclin D1 or Bcl-XL in AML. This observation is reminiscent of the fact that Rapalogs inhibit some but not all of the functions of mTORC1 in normal cells (88). These results also support the rationale behind directly targeting protein synthesis in AML. Indeed, specific inhibition of the eIF4E and eIF4G associations with the 4EGI-1 compound suppresses the formation of the EIF4F translation initiation complex and leads to apoptosis of leukemic cells (59).

A general concern for the future design of novel AML therapies is the role of both PI3K and mTOR activation in the maintenance of the leukemic stem cell compartment. At the moment, no data are available that further elucidate this issue in primary AML cells.

4-4/ Prognostic role of PI3K/AKT activation in AML

An association between AKT phosphorylation and the clinical and biological characteristics of AML has yet to be detected (10). Two studies have shown that PI3K+

DOI: 10.3324/haematol.2009.013797

patients have a poorer prognosis than PI3K- patients (52, 89). In contrast, in our study of a cohort of *de novo* AML patients, we have shown that constitutive PI3K activation is an independent prognostic factor for this cancer, and is associated with better overall and relapse-free survival (10). The reasons for these different findings are not yet clear. One hypothesis for the lower relapse rate of PI3K+ patients is that PI3K may drive immature leukemic cells into the S phase of the cell cycle, increasing their susceptibility to cell cycle-dependent chemotherapies. However, the prognostic value of AKT phosphorylation on Ser473 as opposed to Thr308 may be different. When analyzed by flow cytometry, AKT Thr308 phosphorylation, but not AKT Ser473 phosphorylation, has been found to correlate with high-risk karyotype and shorter overall survival (54).

5/ Disease targets and ligands

The strong rationale behind targeting the PI3K/AKT and mTORC1 pathways in AML has encouraged pharmaceutical firms to develop inhibitors of this signal transduction network (Table 2). We discuss several pharmaceutical inhibitors that selectively target these pathways and have potential in future AML therapies.

5-1/ PK3CD inhibitors

Given that PK3CD expression controls PI3K activity in AML, selective PK3CD inhibitors have been tested *in vitro*. IC87114 is a PK3CD specific inhibitor but is not available for clinical use (9, 60). It has interesting inhibitory effects upon the proliferation and clonogenicity of blast cells. We also anticipate that treatment with PK3CD inhibitors will cause minimal side-effects because of their high specificity. IC87114 does not inhibit the clonogenicity of normal CD34+ cells, and mice lacking PK3CD catalytic activity are viable and manifest defects in B-cells, T-cells and mast cells (90, 91). More potent inhibitors are currently under development by several drug companies such as Piramed, Novartis, and

DOI: 10.3324/haematol.2009.013797

Calistoga. CAL-101 (Calistoga) has just entered Phase I trials for selected relapsed/refractory hematologic malignancies including AML [NCT00710528].

5-2/ mTORC1 inhibitors

Treatment of PTEN-deficient mice with sirolimus restores normal stem cell function and blocks leukemogenesis (43). This suggests that the inhibition of mTORC1 could be used to specifically target LSCs and not affect normal HSCs. Sirolimus and a number of analogues (everolimus, temsirolimus [Torisel, Wyeth]) are currently in Phase II AML trials, alone or with other chemotherapeutics e.g. [NCT00634244, NCT00081874, NCT00544999, NCT00636922, NCT00762632, NCT00819546, NCT00084916]. As a monotherapy, sirolimus induced significant clinical responses in four out of nine patients with either refractory or relapsed AML (56). However, the effects of rapalogs, as previously reported, are limited and only cytostatic *in vitro*. More efficient anti-leukemic activity *in vitro* and *in vivo* will probably be achieved with second generation mTOR inhibitors, now designated as TORK inhibitors (or TORKinhib) (92, 93). These inhibitors are direct competitors of ATP fixation to the catalytic site of mTOR. They inhibit both mTORC1 and mTORC2 complexes and may efficiently inhibit protein translation, in contrast to rapalogs (92, 93).

5-3/ Rationale for inhibiting both PI3K/AKT and mTORC1 as an AML therapy

As described above, there are two principal reasons for targeting both PI3K and mTORC1 as a novel AML therapy. The first is that the mTORC1 pathway is activated independently of PI3K and the second is that mTORC1 inhibition overactivates PI3K (76). In addition, the concomitant inhibition of PI3K and mTORC1 with IC87114 and everolimus, respectively, has additive anti-proliferative effects against AML *in vitro* (76). However, the combination of these compounds is cytostatic and does not induce apoptosis (86). Hence, novel inhibitors of both pathways, such as PI-103, appear to be promising (85-87). As outlined above, PI-103 inhibits blast cell proliferation and induces mitochondrial apoptosis in

DOI: 10.3324/haematol.2009.013797

the LSC compartment (86) and not in normal $CD34^+$ cells. However the bioavailability of PI-103 is not optimal. New dual inhibitors of PI3K and mTOR, such as NVP-BEZ235 (Novartis) (94, 95) which have a better bioavailability (currently in phase I in breast cancer treatment, [NCT00620594]) will soon be tested in AML. Compared with the rapalogs, they have the potential to inhibit both mTORC1 and mTORC2 complexes and may possibly achieve protein synthesis suppression. Due to its facility of administration, NVP-BEZ235 seems a very promising drug which can be used orally in AML, in association with chemotherapy in the induction and consolidation treatment, and probably in the maintenance of minimal residual disease.

5-4/ PDK1 inhibitors

PDK1 phosphorylates AKT on Thr308 and is responsible for the non-PI3K-dependent phosphorylation of other AGC family kinases. Some of those kinases, including PKC, SGK and S6K1, promote cancer progression. PDK1 inhibitors may potentially inhibit cancer progression by blocking the phosphorylation of downstream AGC family kinases including AKT. The staurosporine derivative KRX0601 (7-hydroxystaurosporine, Kyowa Hakko Kogyo) inhibits PDK1 activity [57] and is currently in Phase I clinical trials for AML [NCT00004263 NCT00301938]. *In vitro*, this compound cooperates with cytarabine to decrease AKT phosphorylation in AML (96-98).

5-5/ AKT inhibitors

AKT inhibitors include the family of phosphatidylinositol ether analogues (PIAs) [PMID: 10956212]. PIAs represent a new class of AKT inhibitors that bind to the PH domain of AKT and prevent its translocation to the plasma membrane [PMID: 12553797, PMID: 11300871]. These compounds thereby sensitize leukemic cells to the effects of chemotherapies (99) but they can also activate p38MAPK and AMPK (100, 101). Perifosine (AEterna, Zentaris) is an oral bioactive novel alkylphospholipid that dephosphorylates AKT

DOI: 10.3324/haematol.2009.013797

and ERK 1/2 and causes caspase activation [http://www.aeternazentaris.com/en/page.php?p=60&q=8]. Perifosine induces apoptosis in AML cell lines, reduces the clonogenic activity of AML progenitors but not of normal CD34+ cells, and increases blast cell sensitivity to etoposide (102). In blast cells, perifosine may increase the response of tumour necrosis factor-related apoptosis-inducing ligand (TRAIL) cytotoxicity via the upregulation of TRAIL-R2 receptor expression and suppression of cFLIP expression (103). Perifosine is currently in a phase II trial for AML [NCT00391560]. Deguelin (Alexis Biochemicals) is a naturally occurring rotenoid that inhibits AKT activity through an unknown mechanism and has been shown to increase AML sensitivity to Cytarabine (104). Other AKT inhibitors that are currently in development include AEZS-127 (Aeterna, Zentaris), which is an alkylphospholipid similar to perifosine, and triciribine (VioQuest), all of which have been tested in phase I trials for relapsed/refractory hematologic malignancies [NCT00642031].

5-6/ SHIP1 agonists

Activation of the phosphatases that control PIP3 degradation might also inhibit PI3K. The meroterpenoid Pelorol and two structural analogues, AQX-016A (Aquinox) and AQX-MN100 (Aquinox) exhibit SHIP1 activating activity in macrophages and mast cells (105). As SHIP1 expression is restricted to the hematopoietic cells, these inhibitors might also specifically increase the activity of PI3K inhibitors in the hematopoietic system.

5-7/ Inhibition of the IGF-1/IGF-1R system

Neutralizing anti-IGF-1R antibodies or inhibitors of IGF-1R kinase activity, such as NVP-AEW541 (Novartis), inhibit the IGF-1/IGF-1R loop (75). NVP-AEW541 induces apoptosis and sensitizes blast cells to etoposide treatment (61, 75), whereas AVE-1642 (Sanofi-Aventis) is an anti IGF-1R antibody. However, chronic inhibition of the IGF-1/IGF-1R system is likely to induce unwanted side-effects as it could influence glucose metabolism.

DOI: 10.3324/haematol.2009.013797

Inhibition of IGF-1R could be effective and tolerated for short-term periods but is most likely not suitable for maintenance therapy.

5-8/ Multiple kinase inhibitors

The use of inhibitors targeting different signalling pathways might override drug resistance in AML. The multiple kinase inhibitor KP372-1 (QLT) directly inhibits the kinase activity of AKT, PDK1 and FLT3, induces blast cells apoptosis and inhibits the colony-forming ability of AML samples (106). BAG956 (Novartis) is the first dual PI3K/PDK1 inhibitor that enhances the inhibitory effects of midostaurin (Novartis), an inhibitor of mutant FLT3 (107) that is currently in Phase III trials for AML [NCT00651261]. BAG956 also has synergistic effects with rapamycin in different AML cell lines (107).

5-9/ Heat shock protein inhibitors

AKT is a target for heat shock proteins (HSP), and is stabilized by these interactions. HSP-90 is a molecular chaperone that interacts with the AKT kinase domain and positively regulates its activity (108). The HSP90 inhibitor tanespimycin (Bristol-Myers Squibb), might therefore be useful in the treatment of PI3K+ AML, in part through the destabilization of other client kinases, including FLT3, RAF and AKT (109, 110).

6/ Conclusions

PI3K is frequently activated in AML, whereas mTORC1 is active in all cases of this disease. Both pathways contribute to the proliferation of blast cells and leukemic progenitors. However, the reasons why the inhibition of each pathway alone does not induce significant apoptosis is of some interest. The interactions between PI3K and mTOR are complex in AML and provide a rationale for simultaneous inhibition of both pathways. The development of dual PI3K and mTOR inhibitors seems promising in this respect.

DOI: 10.3324/haematol.2009.013797

New mechanisms of resistance to rapalogs have now been described in AML. Among these, the fact that protein translation escapes mTORC1 control and that 4E-BP1 phosphorylation is controlled by the PIM2 kinase which is constantly expressed in AML, represent potential new targets. Also, second generation inhibitors that have the capacity to inhibit both the mTORC1 and 2 complexes will need to be tested in AML *in vitro* to determine whether they can achieve cap-dependent mRNA translation inhibition. Another aspect of this type of research will be to better understand the mechanisms leading to constitutive mTORC1 activation in blast cells independently of PI3K, with particular attention needed on the role of the amino acid and energetic LKB1/AMPK signalling pathways.

The development of compounds directed against PI3K/AKT, mTORC1 and mTORC2 and their modulators as novel and potent agents for treating AML is expanding. Nevertheless, a major issue with these compounds is whether they will be sufficiently specific for the deregulated pathway in the leukemic cell and particularly in the LSCs, in order to avoid damaging normal cells. Other challenging issues include the complexity of the signalling network that comprises multitude of regulatory loops (111), and the existence of the hematopoietic niche providing an external environment, which could influence the fate and the signalling pathways of AML blasts (112). Finally, the concept of the leukemic stem cell receiving important signals from the hematopoietic niche, that support self-renewal, could explain the chemoresistance found in this disease (113). Many of the future efforts described herein should thus lead to a better comprehension of the role of PI3K/AKT and mTOR pathways in the biology of the LSCs.

DOI: 10.3324/haematol.2009.013797

Authorship and Disclosures

SP and DB designed and wrote the manuscript. NC, JT, VB, PC, LW, AG performed the experiments. DB, PM and CL supervised the work. All authors do not have potential financial interests to disclose.

Aknowledgments

We thank Dr Yasmin Reyal and Dr David Taussig for reading the manuscript.

Table 1. Mechanisms potentially contributing to PI3K/AKT activation in AML.

Table 2. Therapeutic targets in the PI3K/AKT and mTOR signalling pathways in AML.

Figure 1. The PI3K/AKT signalling pathway.

An activated tyrosine kinase receptor (RTK) recruits adaptators such as Gab2 or IRS family proteins, which bind to the regulatory p85 subunit of PI3K. The latter activates the catalytic p110alpha, beta and delta subunits of PI3K. Activated PI3K complex transforms PI(4,5)P2 into PI(3,4,5)P3. The latter recruits PDK1 and AKT to the plasma membrane where AKT is phosphorylated by PDK1 on Thr308. PDK2, which is mTORC2, phosphorylates AKT on Ser473. Fully activated AKT modulates several substrates important for cell survival, cell cycle and cell growth.

DOI: 10.3324/haematol.2009.013797

Figure 2. Regulation of mTORC1 activation downstream of AKT and interactions between mTORC1 and PI3K.

Active AKT inhibits TSC2 activity through direct phosphorylation. TSC2 functions in association with the putative TSC1 to inactivate the small G protein Rheb. AKT–driven TSC1/TSC2 inactivation allows Rheb to accumulate in a GTP-bound state. Rheb-GTP activates mTORC1 by inhibiting FKBP38 (114). mTORC1 phosphorylates p70S6 kinase which has a role in mRNA translation and which mediates a negative feedback to AKT through IRS-1 degradation. MTORC2 complex phosphorylates AKT on Ser473.

DOI: 10.3324/haematol.2009.013797

Abbreviations

4EBP-1	4E binding protein
CMP	common myeloid progenitor
EPO	erythropoietin
ERK	extracellular regulated kinase
FKBP12	FK506 binding protein of molecular mass 12 kDa
Flt3-ITD	fms-like tyrosine kinase
FOXO	Forkhead box o protein
Gab1/Gab2	grab2-associated binder 1/2
GSK-3b	glycogen-synthase kinase-3beta
HSC	hematopoietic stem cell
HSP	heat shock protein
IGF1	insulin growth factor 1
ILK	integrin-linked kinase
IRS-1	insulin receptor substrate-1
LSC	leukemic stem cells
mSin1	mSAPK interacting protein1
mTOR	mammalian target of rapamycin
mTORC2	mTOR/Rictor (rapamycin-insensitive companion of mTOR/mLST8 complex)
PDK1	phosphoinositide-dependent protein kinase-1
PH	plekstrin homology
PHLPP	PH domain leucin-rich repeat protein phosphatase
PI3K	phospho-inositide 3 kinase
PIP2	phosphatidyl inositol bisphosphate
PIP3	phosphatidyl inositol trisphosphate
PKB	protein kinase B
PP2A	protein phosphatase 2A
PTEN	phosphatase and tensin homologue deleted on chromosome 10
Raptor	regulatory associated protein of mTOR
Rheb	ras homologue-enriched brain
Rictor	rapamycin insensitive companion of mTOR
ROS	Reactive oxygen species
SHIP-1	SH2 domain containing inositol 5'phosphatase
TK	tyrosine kinase
TSC2 or hamartin	tuberous sclerosis 2
VEGF	vascular endothelial growth factor

References

1. Steffen B, Muller-Tidow C, Schwable J, Berdel WE, Serve H. The molecular pathogenesis of acute myeloid leukemia. Crit Rev Oncol Hematol. 2005 Nov;56(2):195-221.
2. Rowe JM. Why is clinical progress in acute myelogenous leukemia so slow? Best Pract Res Clin Haematol. 2008 Mar;21(1):1-3.
3. Gilliland DG. Targeted therapies in myeloid leukemias. Ann Hematol. 2004;83 Suppl 1:S75-6.
4. Jordan CT. Searching for leukemia stem cells--not yet the end of the road? Cancer Cell. 2006 Oct;10(4):253-4.
5. Jordan CT. The leukemic stem cell. Best Pract Res Clin Haematol. 2007 Mar;20(1):13-8.
6. Hope KJ, Jin L, Dick JE. Acute myeloid leukemia originates from a hierarchy of leukemic stem cell classes that differ in self-renewal capacity. Nat Immunol. 2004 Jul;5(7):738-43.
7. Gilliland DG. Molecular genetics of human leukemias: new insights into therapy. Semin Hematol. 2002 Oct;39(4 Suppl 3):6-11.
8. Wu J, Wong WW, Khosravi F, Minden MD, Penn LZ. Blocking the Raf/MEK/ERK pathway sensitizes acute myelogenous leukemia cells to lovastatin-induced apoptosis. Cancer Res. 2004 Sep 15;64(18):6461-8.
9. Sujobert P, Bardet V, Cornillet-Lefebvre P, Hayflick JS, Prie N, Verdier F, et al. Essential role for the p110delta isoform in phosphoinositide 3-kinase activation and cell proliferation in acute myeloid leukemia. Blood. 2005 Aug 1;106(3):1063-6.
10. Tamburini J, Elie C, Bardet V, Chapuis N, Park S, Broet P, et al. Constitutive phosphoinositide 3-kinase/Akt activation represents a favorable prognostic factor in de novo acute myelogenous leukemia patients. Blood. 2007 Aug 1;110(3):1025-8.
11. Dos Santos C, Recher C, Demur C, Payrastre B. [The PI3K/Akt/mTOR pathway: a new therapeutic target in the treatment of acute myeloid leukemia]. Bull Cancer. 2006 May 1;93(5):445-7.
12. Steelman LS, Abrams SL, Whelan J, Bertrand FE, Ludwig DE, Basecke J, et al. Contributions of the Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways to leukemia. Leukemia. 2008 Apr;22(4):686-707.
13. McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EW, Chang F, et al. Roles of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta. 2007 Aug;1773(8):1263-84.
14. De Toni F, Racaud-Sultan C, Chicanne G, Mas VM, Cariven C, Mesange F, et al. A crosstalk between the Wnt and the adhesion-dependent signaling pathways governs the chemosensitivity of acute myeloid leukemia. Oncogene. 2006 May 25;25(22):3113-22.
15. Petropoulos K, Arseni N, Schessl C, Stadler CR, Rawat VP, Deshpande AJ, et al. A novel role for Lef-1, a central transcription mediator of Wnt signaling, in leukemogenesis. J Exp Med. 2008 Mar 17;205(3):515-22.
16. Valencia A, Roman-Gomez J, Cervera J, Such E, Barragan E, Bolufer P, et al. Wnt signaling pathway is epigenetically regulated by methylation of Wnt antagonists in acute myeloid leukemia. Leukemia. 2009 Apr 23.
17. Griessinger E, Imbert V, Lagadec P, Gonthier N, Dubreuil P, Romanelli A, et al. AS602868, a dual inhibitor of IKK2 and FLT3 to target AML cells. Leukemia. 2007 May;21(5):877-85.

18. Guzman ML, Neering SJ, Upchurch D, Grimes B, Howard DS, Rizzieri DA, et al. Nuclear factor-kappaB is constitutively activated in primitive human acute myelogenous leukemia cells. Blood. 2001 Oct 15;98(8):2301-7.
19. Guzman ML, Swiderski CF, Howard DS, Grimes BA, Rossi RM, Szilvassy SJ, et al. Preferential induction of apoptosis for primary human leukemic stem cells. Proc Natl Acad Sci U S A. 2002 Dec 10;99(25):16220-5.
20. Baumgartner B, Weber M, Quirling M, Fischer C, Page S, Adam M, et al. Increased IkappaB kinase activity is associated with activated NF-kappaB in acute myeloid blasts. Leukemia. 2002 Oct;16(10):2062-71.
21. Engelman JA. Targeting PI3K signalling in cancer: opportunities, challenges and limitations. Nat Rev Cancer. 2009 Aug;9(8):550-62.
22. Yuan TL, Cantley LC. PI3K pathway alterations in cancer: variations on a theme. Oncogene. 2008 Sep 18;27(41):5497-510.
23. Hawkins PT, Anderson KE, Davidson K, Stephens LR. Signalling through Class I PI3Ks in mammalian cells. Biochem Soc Trans. 2006 Nov;34(Pt 5):647-62.
24. Manning BD, Cantley LC. AKT/PKB signaling: navigating downstream. Cell. 2007 Jun 29;129(7):1261-74.
25. Greer EL, Brunet A. FOXO transcription factors at the interface between longevity and tumor suppression. Oncogene. 2005 Nov 14;24(50):7410-25.
26. Scheijen B, Ngo HT, Kang H, Griffin JD. FLT3 receptors with internal tandem duplications promote cell viability and proliferation by signaling through Foxo proteins. Oncogene. 2004 Apr 22;23(19):3338-49.
27. Leevers SJ, Vanhaesebroeck B, Waterfield MD. Signalling through phosphoinositide 3-kinases: the lipids take centre stage. Curr Opin Cell Biol. 1999 Apr;11(2):219-25.
28. Stambolic V, Suzuki A, de la Pompa JL, Brothers GM, Mirtsos C, Sasaki T, et al. Negative regulation of PKB/Akt-dependent cell survival by the tumor suppressor PTEN. Cell. 1998 Oct 2;95(1):29-39.
29. Gao T, Furnari F, Newton AC. PHLPP: a phosphatase that directly dephosphorylates Akt, promotes apoptosis, and suppresses tumor growth. Mol Cell. 2005 Apr 1;18(1):13-24.
30. Trotman LC, Alimonti A, Scaglioni PP, Koutcher JA, Cordon-Cardo C, Pandolfi PP. Identification of a tumour suppressor network opposing nuclear Akt function. Nature. 2006 May 25;441(7092):523-7.
31. Long X, Lin Y, Ortiz-Vega S, Yonezawa K, Avruch J. Rheb binds and regulates the mTOR kinase. Curr Biol. 2005 Apr 26;15(8):702-13.
32. Inoki K, Li Y, Zhu T, Wu J, Guan KL. TSC2 is phosphorylated and inhibited by Akt and suppresses mTOR signalling. Nat Cell Biol. 2002 Sep;4(9):648-57.
33. Ruvinsky I, Meyuhas O. Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. Trends Biochem Sci. 2006 Jun;31(6):342-8.
34. Holz MK, Ballif BA, Gygi SP, Blenis J. mTOR and S6K1 mediate assembly of the translation preinitiation complex through dynamic protein interchange and ordered phosphorylation events. Cell. 2005 Nov 18;123(4):569-80.
35. De Benedetti A, Graff JR. eIF-4E expression and its role in malignancies and metastases. Oncogene. 2004 Apr 19;23(18):3189-99.
36. Harrington LS, Findlay GM, Gray A, Tolkacheva T, Wigfield S, Rebholz H, et al. The TSC1-2 tumor suppressor controls insulin-PI3K signaling via regulation of IRS proteins. J Cell Biol. 2004 Jul 19;166(2):213-23.
37. Kwon J, Stephan S, Mukhopadhyay A, Muders MH, Dutta SK, Lau JS, et al. Insulin receptor substrate-2 mediated insulin-like growth factor-I receptor

overexpression in pancreatic adenocarcinoma through protein kinase Cdelta. Cancer Res. 2009 Feb 15;69(4):1350-7.
38. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science. 2005 Feb 18;307(5712):1098-101.
39. Garcia-Martinez JM, Alessi DR. mTOR complex 2 (mTORC2) controls hydrophobic motif phosphorylation and activation of serum- and glucocorticoid-induced protein kinase 1 (SGK1). Biochem J. 2008 Dec 15;416(3):375-85.
40. Guertin DA, Stevens DM, Thoreen CC, Burds AA, Kalaany NY, Moffat J, et al. Ablation in mice of the mTORC components raptor, rictor, or mLST8 reveals that mTORC2 is required for signaling to Akt-FOXO and PKCalpha, but not S6K1. Dev Cell. 2006 Dec;11(6):859-71.
41. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, et al. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell. 2006 Apr 21;22(2):159-68.
42. Peterson TR, Laplante M, Thoreen CC, Sancak Y, Kang SA, Kuehl WM, et al. DEPTOR is an mTOR inhibitor frequently overexpressed in multiple myeloma cells and required for their survival. Cell. 2009 May 29;137(5):873-86.
43. Yilmaz OH, Valdez R, Theisen BK, Guo W, Ferguson DO, Wu H, et al. Pten dependence distinguishes haematopoietic stem cells from leukaemia-initiating cells. Nature. 2006 May 25;441(7092):475-82.
44. Zhang J, Grindley JC, Yin T, Jayasinghe S, He XC, Ross JT, et al. PTEN maintains haematopoietic stem cells and acts in lineage choice and leukaemia prevention. Nature. 2006 May 25;441(7092):518-22.
45. Myklebust JH, Blomhoff HK, Rusten LS, Stokke T, Smeland EB. Activation of phosphatidylinositol 3-kinase is important for erythropoietin-induced erythropoiesis from CD34(+) hematopoietic progenitor cells. Exp Hematol. 2002 Sep;30(9):990-1000.
46. Bouscary D, Pene F, Claessens YE, Muller O, Chretien S, Fontenay-Roupie M, et al. Critical role for PI 3-kinase in the control of erythropoietin-induced erythroid progenitor proliferation. Blood. 2003 May 1;101(9):3436-43.
47. Buitenhuis M, Verhagen LP, van Deutekom HW, Castor A, Verploegen S, Koenderman L, et al. Protein kinase B (c-akt) regulates hematopoietic lineage choice decisions during myelopoiesis. Blood. 2008 Jan 1;111(1):112-21.
48. Chen C, Liu Y, Liu R, Ikenoue T, Guan KL, Liu Y, et al. TSC-mTOR maintains quiescence and function of hematopoietic stem cells by repressing mitochondrial biogenesis and reactive oxygen species. J Exp Med. 2008 Sep 29;205(10):2397-408.
49. Gan B, Sahin E, Jiang S, Sanchez-Aguilera A, Scott KL, Chin L, et al. mTORC1-dependent and -independent regulation of stem cell renewal, differentiation, and mobilization. Proc Natl Acad Sci U S A. 2008 Dec 9;105(49):19384-9.
50. Tothova Z, Kollipara R, Huntly BJ, Lee BH, Castrillon DH, Cullen DE, et al. FoxOs are critical mediators of hematopoietic stem cell resistance to physiologic oxidative stress. Cell. 2007 Jan 26;128(2):325-39.
51. Xu Q, Simpson SE, Scialla TJ, Bagg A, Carroll M. Survival of acute myeloid leukemia cells requires PI3 kinase activation. Blood. 2003 Aug 1;102(3):972-80.
52. Min YH, Eom JI, Cheong JW, Maeng HO, Kim JY, Jeung HK, et al. Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable. Leukemia. 2003 May;17(5):995-7.

53. Grandage VL, Gale RE, Linch DC, Khwaja A. 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. 2005 Apr;19(4):586-94.
54. Gallay N, Dos Santos C, Cuzin L, Bousquet M, Simmonet Gouy V, Chaussade C, et al. 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. 2009 Jan 22.
55. Bardet V, Tamburini J, Ifrah N, Dreyfus F, Mayeux P, Bouscary D, et al. Single cell analysis of phosphoinositide 3-kinase/Akt and ERK activation in acute myeloid leukemia by flow cytometry. Haematologica. 2006 Jun;91(6):757-64.
56. Recher C, Beyne-Rauzy O, Demur C, Chicanne G, Dos Santos C, Mas VM, et al. Antileukemic activity of rapamycin in acute myeloid leukemia. Blood. 2005 Mar 15;105(6):2527-34.
57. Recher C, Dos Santos C, Demur C, Payrastre B. mTOR, a new therapeutic target in acute myeloid leukemia. Cell Cycle. 2005 Nov;4(11):1540-9.
58. Xu Q, Thompson JE, Carroll M. mTOR regulates cell survival after etoposide treatment in primary AML cells. Blood. 2005 Dec 15;106(13):4261-8.
59. Tamburini J, Green AS, Bardet V, Chapuis N, Park S, Willems L, et al. Protein synthesis is resistant to rapamycin and constitutes a promising therapeutic target in acute myeloid leukemia. Blood. 2009 Aug 20;114(8):1618-27.
60. Billottet C, Grandage VL, Gale RE, Quattropani A, Rommel C, Vanhaesebroeck B, et al. 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. 2006 Oct 26;25(50):6648-59.
61. Doepfner KT, Spertini O, Arcaro A. Autocrine insulin-like growth factor-I signaling promotes growth and survival of human acute myeloid leukemia cells via the phosphoinositide 3-kinase/Akt pathway. Leukemia. 2007 Sep;21(9):1921-30.
62. Billottet C, Banerjee L, Vanhaesebroeck B, Khwaja A. Inhibition of class I phosphoinositide 3-kinase activity impairs proliferation and triggers apoptosis in acute promyelocytic leukemia without affecting atra-induced differentiation. Cancer Res. 2009 Feb 1;69(3):1027-36.
63. Kang S, Denley A, Vanhaesebroeck B, Vogt PK. Oncogenic transformation induced by the p110beta, -gamma, and -delta isoforms of class I phosphoinositide 3-kinase. Proc Natl Acad Sci U S A. 2006 Jan 31;103(5):1289-94.
64. Kok K, Nock GE, Verrall EA, Mitchell MP, Hommes DW, Peppelenbosch MP, et al. Regulation of p110delta PI 3-kinase gene expression. PLoS One. 2009;4(4):e5145.
65. Bousquet M, Recher C, Queleen C, Demur C, Payrastre B, Brousset P. Assessment of somatic mutations in phosphatidylinositol 3-kinase gene in human lymphoma and acute leukaemia. Br J Haematol. 2005 Nov;131(3):411-3.
66. Cornillet-Lefebvre P, Cuccuini W, Bardet V, Tamburini J, Gillot L, Ifrah N, et al. Constitutive phosphoinositide 3-kinase activation in acute myeloid leukemia is not due to p110delta mutations. Leukemia. 2006 Feb;20(2):374-6.
67. Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, et al. Patterns of somatic mutation in human cancer genomes. Nature. 2007 Mar 8;446(7132):153-8.
68. Carpten JD, Faber AL, Horn C, Donoho GP, Briggs SL, Robbins CM, et al. A transforming mutation in the pleckstrin homology domain of AKT1 in cancer. Nature. 2007 Jul 26;448(7152):439-44.

69. Tibes R, Kornblau SM, Qiu Y, Mousses SM, Robbins C, Moses T, et al. PI3K/AKT pathway activation in acute myeloid leukaemias is not associated with AKT1 pleckstrin homology domain mutation. Br J Haematol. 2008 Feb;140(3):344-7.
70. Luo JM, Yoshida H, Komura S, Ohishi N, Pan L, Shigeno K, et al. Possible dominant-negative mutation of the SHIP gene in acute myeloid leukemia. Leukemia. 2003 Jan;17(1):1-8.
71. Cheong JW, Eom JI, Maeng HY, Lee ST, Hahn JS, Ko YW, et al. Phosphatase and tensin homologue phosphorylation in the C-terminal regulatory domain is frequently observed in acute myeloid leukaemia and associated with poor clinical outcome. Br J Haematol. 2003 Aug;122(3):454-6.
72. Metzner A, Precht C, Fehse B, Fiedler W, Stocking C, Gunther A, et al. Reduced proliferation of CD34(+) cells from patients with acute myeloid leukemia after gene transfer of INPP5D. Gene Ther. 2009 Apr;16(4):570-3.
73. Pedersen M, Ronnstrand L, Sun J. The c-Kit/D816V mutation eliminates the differences in signal transduction and biological responses between two isoforms of c-Kit. Cell Signal. 2008 Nov 17.
74. Sun J, Pedersen M, Ronnstrand L. Gab2 is involved in differential phosphoinositide 3-kinase signaling by two splice forms of c-Kit. J Biol Chem. 2008 Oct 10;283(41):27444-51.
75. Tazzari PL, Tabellini G, Bortul R, Papa V, Evangelisti C, Grafone T, et al. The insulin-like growth factor-I receptor kinase inhibitor NVP-AEW541 induces apoptosis in acute myeloid leukemia cells exhibiting autocrine insulin-like growth factor-I secretion. Leukemia. 2007 May;21(5):886-96.
76. Tamburini J, Chapuis N, Bardet V, Park S, Sujobert P, Willems L, et al. 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. 2008 Jan 1;111(1):379-82.
77. List AF, Glinsmann-Gibson B, Stadheim C, Meuillet EJ, Bellamy W, Powis G. Vascular endothelial growth factor receptor-1 and receptor-2 initiate a phosphatidylinositide 3-kinase-dependent clonogenic response in acute myeloid leukemia cells. Exp Hematol. 2004 Jun;32(6):526-35.
78. Wakabayashi M, Miwa H, Shikami M, Hiramatsu A, Ikai T, Tajima E, et al. Autocrine pathway of angiopoietins-Tie2 system in AML cells: association with phosphatidyl-inositol 3 kinase. Hematol J. 2004;5(4):353-60.
79. Matsunaga T, Takemoto N, Sato T, Takimoto R, Tanaka I, Fujimi A, et al. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. Nat Med. 2003 Sep;9(9):1158-65.
80. Zeng Z, Sarbassov dos D, Samudio IJ, Yee KW, Munsell MF, Ellen Jackson C, et al. Rapamycin derivatives reduce mTORC2 signaling and inhibit AKT activation in AML. Blood. 2007 Apr 15;109(8):3509-12.
81. Tabe Y, Jin L, Tsutsumi-Ishii Y, Xu Y, McQueen T, Priebe W, et al. Activation of integrin-linked kinase is a critical prosurvival pathway induced in leukemic cells by bone marrow-derived stromal cells. Cancer Res. 2007 Jan 15;67(2):684-94.
82. Dos Santos C, Demur C, Bardet V, Prade-Houdellier N, Payrastre B, Recher C. A critical role for Lyn in acute myeloid leukemia. Blood. 2008 Feb 15;111(4):2269-79.

83. Chow S, Minden MD, Hedley DW. Constitutive phosphorylation of the S6 ribosomal protein via mTOR and ERK signaling in the peripheral blasts of acute leukemia patients. Exp Hematol. 2006 Sep;34(9):1183-91.
84. Zhong D, Liu X, Khuri FR, Sun SY, Vertino PM, Zhou W. LKB1 is necessary for Akt-mediated phosphorylation of proapoptotic proteins. Cancer Res. 2008 Sep 15;68(18):7270-7.
85. Knight ZA, Gonzalez B, Feldman ME, Zunder ER, Goldenberg DD, Williams O, et al. A pharmacological map of the PI3-K family defines a role for p110alpha in insulin signaling. Cell. 2006 May 19;125(4):733-47.
86. Park S, Chapuis N, Bardet V, Tamburini J, Gallay N, Willems L, et al. PI-103, a dual inhibitor of Class IA phosphatidylinositide 3-kinase and mTOR, has antileukemic activity in AML. Leukemia. 2008 Sep;22(9):1698-706.
87. Kojima K, Shimanuki M, Shikami M, Samudio IJ, Ruvolo V, Corn P, et al. 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. 2008 Sep;22(9):1728-36.
88. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, et al. An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem. 2009 Mar 20;284(12):8023-32.
89. Kornblau SM, Womble M, Qiu YH, Jackson CE, Chen W, Konopleva M, et al. Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia. Blood. 2006 Oct 1;108(7):2358-65.
90. Ali K, Bilancio A, Thomas M, Pearce W, Gilfillan AM, Tkaczyk C, et al. Essential role for the p110delta phosphoinositide 3-kinase in the allergic response. Nature. 2004 Oct 21;431(7011):1007-11.
91. Okkenhaug K, Ali K, Vanhaesebroeck B. Antigen receptor signalling: a distinctive role for the p110delta isoform of PI3K. Trends Immunol. 2007 Feb;28(2):80-7.
92. Feldman ME, Apsel B, Uotila A, Loewith R, Knight ZA, Ruggero D, et al. Active-site inhibitors of mTOR target rapamycin-resistant outputs of mTORC1 and mTORC2. PLoS Biol. 2009 Feb 10;7(2):e38.
93. Garcia-Martinez JM, Moran J, Clarke RG, Gray A, Cosulich SC, Chresta CM, et al. Ku-0063794 is a specific inhibitor of the mammalian target of rapamycin (mTOR). Biochem J. 2009 Jul 1;421(1):29-42.
94. Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, et al. 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. Mol Cancer Ther. 2008 Jul;7(7):1851-63.
95. Serra V, Markman B, Scaltriti M, Eichhorn PJ, Valero V, Guzman M, et al. NVP-BEZ235, a dual PI3K/mTOR inhibitor, prevents PI3K signaling and inhibits the growth of cancer cells with activating PI3K mutations. Cancer Res. 2008 Oct 1;68(19):8022-30.
96. Sato S, Fujita N, Tsuruo T. Interference with PDK1-Akt survival signaling pathway by UCN-01 (7-hydroxystaurosporine). Oncogene. 2002 Mar 7;21(11):1727-38.
97. Komander D, Kular GS, Bain J, Elliott M, Alessi DR, Van Aalten DM. Structural basis for UCN-01 (7-hydroxystaurosporine) specificity and PDK1 (3-phosphoinositide-dependent protein kinase-1) inhibition. Biochem J. 2003 Oct 15;375(Pt 2):255-62.

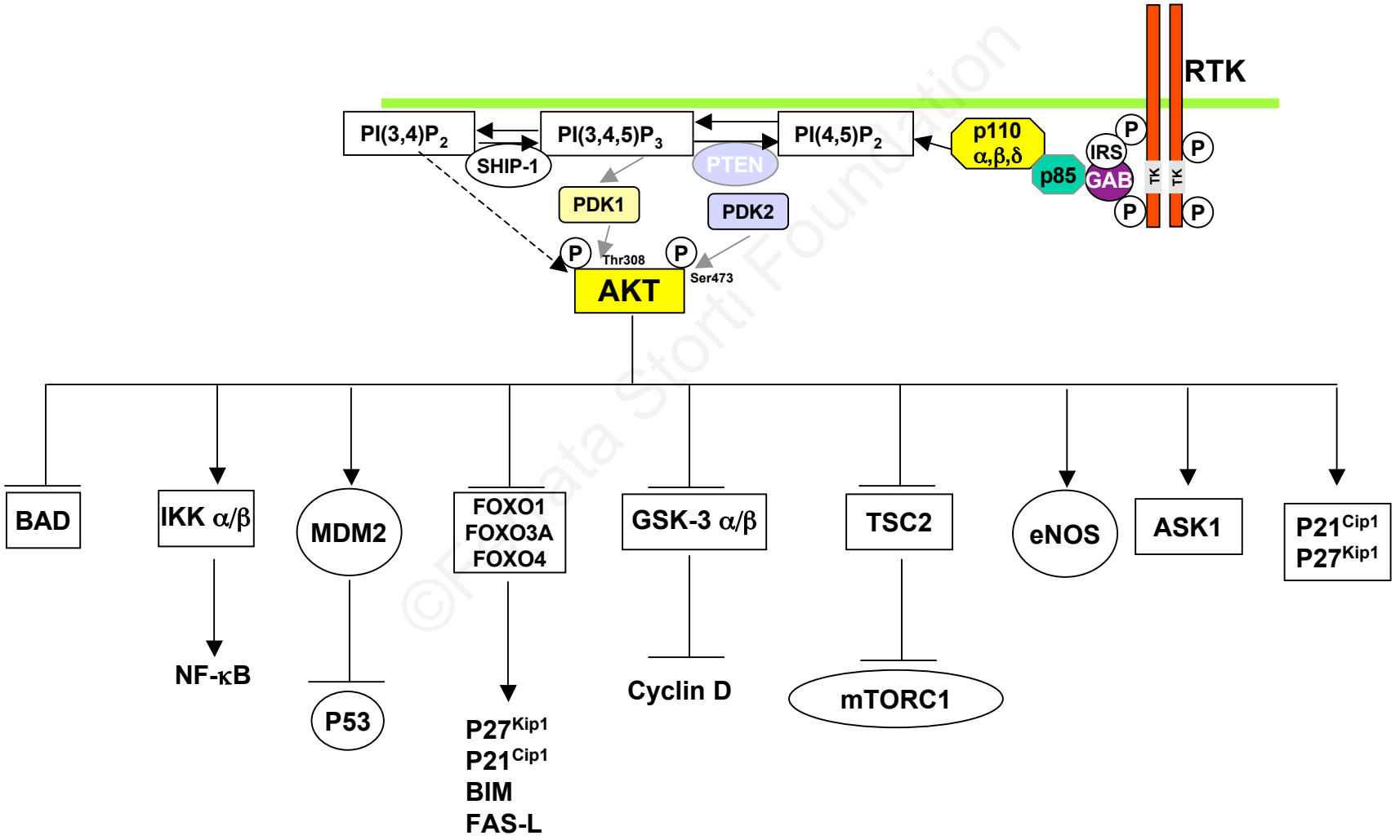
98. Sampath D, Cortes J, Estrov Z, Du M, Shi Z, Andreeff M, et al. Pharmacodynamics of cytarabine alone and in combination with 7-hydroxystaurosporine (UCN-01) in AML blasts in vitro and during a clinical trial. Blood. 2006 Mar 15;107(6):2517-24.
99. Gills JJ, Dennis PA. The development of phosphatidylinositol ether lipid analogues as inhibitors of the serine/threonine kinase, Akt. Expert Opin Investig Drugs. 2004 Jul;13(7):787-97.
100. Gills JJ, Castillo SS, Zhang C, Petukhov PA, Memmott RM, Hollingshead M, et al. Phosphatidylinositol ether lipid analogues that inhibit AKT also independently activate the stress kinase, p38alpha, through MKK3/6-independent and -dependent mechanisms. J Biol Chem. 2007 Sep 14;282(37):27020-9.
101. Memmott RM, Gills JJ, Hollingshead M, Powers MC, Chen Z, Kemp B, et al. Phosphatidylinositol ether lipid analogues induce AMP-activated protein kinase-dependent death in LKB1-mutant non small cell lung cancer cells. Cancer Res. 2008 Jan 15;68(2):580-8.
102. Papa V, Tazzari PL, Chiarini F, Cappellini A, Ricci F, Billi AM, et al. Proapoptotic activity and chemosensitizing effect of the novel Akt inhibitor perifosine in acute myelogenous leukemia cells. Leukemia. 2008 Jan;22(1):147-60.
103. Tazzari PL, Tabellini G, Ricci F, Papa V, Bortul R, Chiarini F, et al. Synergistic proapoptotic activity of recombinant TRAIL plus the Akt inhibitor Perifosine in acute myelogenous leukemia cells. Cancer Res. 2008 Nov 15;68(22):9394-403.
104. Bortul R, Tazzari PL, Billi AM, Tabellini G, Mantovani I, Cappellini A, et al. Deguelin, A PI3K/AKT inhibitor, enhances chemosensitivity of leukaemia cells with an active PI3K/AKT pathway. Br J Haematol. 2005 Jun;129(5):677-86.
105. Ong CJ, Ming-Lum A, Nodwell M, Ghanipour A, Yang L, Williams DE, et al. Small-molecule agonists of SHIP1 inhibit the phosphoinositide 3-kinase pathway in hematopoietic cells. Blood. 2007 Sep 15;110(6):1942-9.
106. Zeng Z, Samudio IJ, Zhang W, Estrov Z, Pelicano H, Harris D, et al. 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 Res. 2006 Apr 1;66(7):3737-46.
107. Weisberg E, Banerji L, Wright RD, Barrett R, Ray A, Moreno D, et al. Potentiation of antileukemic therapies by the dual PI3K/PDK-1 inhibitor, BAG956: effects on BCR-ABL- and mutant FLT3-expressing cells. Blood. 2008 Apr 1;111(7):3723-34.
108. Zhang R, Luo D, Miao R, Bai L, Ge Q, Sessa WC, et al. Hsp90-Akt phosphorylates ASK1 and inhibits ASK1-mediated apoptosis. Oncogene. 2005 Jun 2;24(24):3954-63.
109. George P, Bali P, Cohen P, Tao J, Guo F, Sigua C, et al. Cotreatment with 17-allylamino-demethoxygeldanamycin and FLT-3 kinase inhibitor PKC412 is highly effective against human acute myelogenous leukemia cells with mutant FLT-3. Cancer Res. 2004 May 15;64(10):3645-52.
110. George P, Bali P, Annavarapu S, Scuto A, Fiskus W, Guo F, et al. Combination of the histone deacetylase inhibitor LBH589 and the hsp90 inhibitor 17-AAG is highly active against human CML-BC cells and AML cells with activating mutation of FLT-3. Blood. 2005 Feb 15;105(4):1768-76.
111. Liu P, Cheng H, Roberts TM, Zhao JJ. Targeting the phosphoinositide 3-kinase pathway in cancer. Nat Rev Drug Discov. 2009 Aug;8(8):627-44.
112. Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. Blood. 2009 Aug 6;114(6):1150-7.

DOI: 10.3324/haematol.2009.013797

113. Weisberg E, Barrett R, Liu Q, Stone R, Gray N, Griffin JD. FLT3 inhibition and mechanisms of drug resistance in mutant FLT3-positive AML. Drug Resist Updat. 2009 Jun;12(3):81-9.
114. Bai X, Ma D, Liu A, Shen X, Wang QJ, Liu Y, et al. Rheb activates mTOR by antagonizing its endogenous inhibitor, FKBP38. Science. 2007 Nov 9;318(5852):977-80.

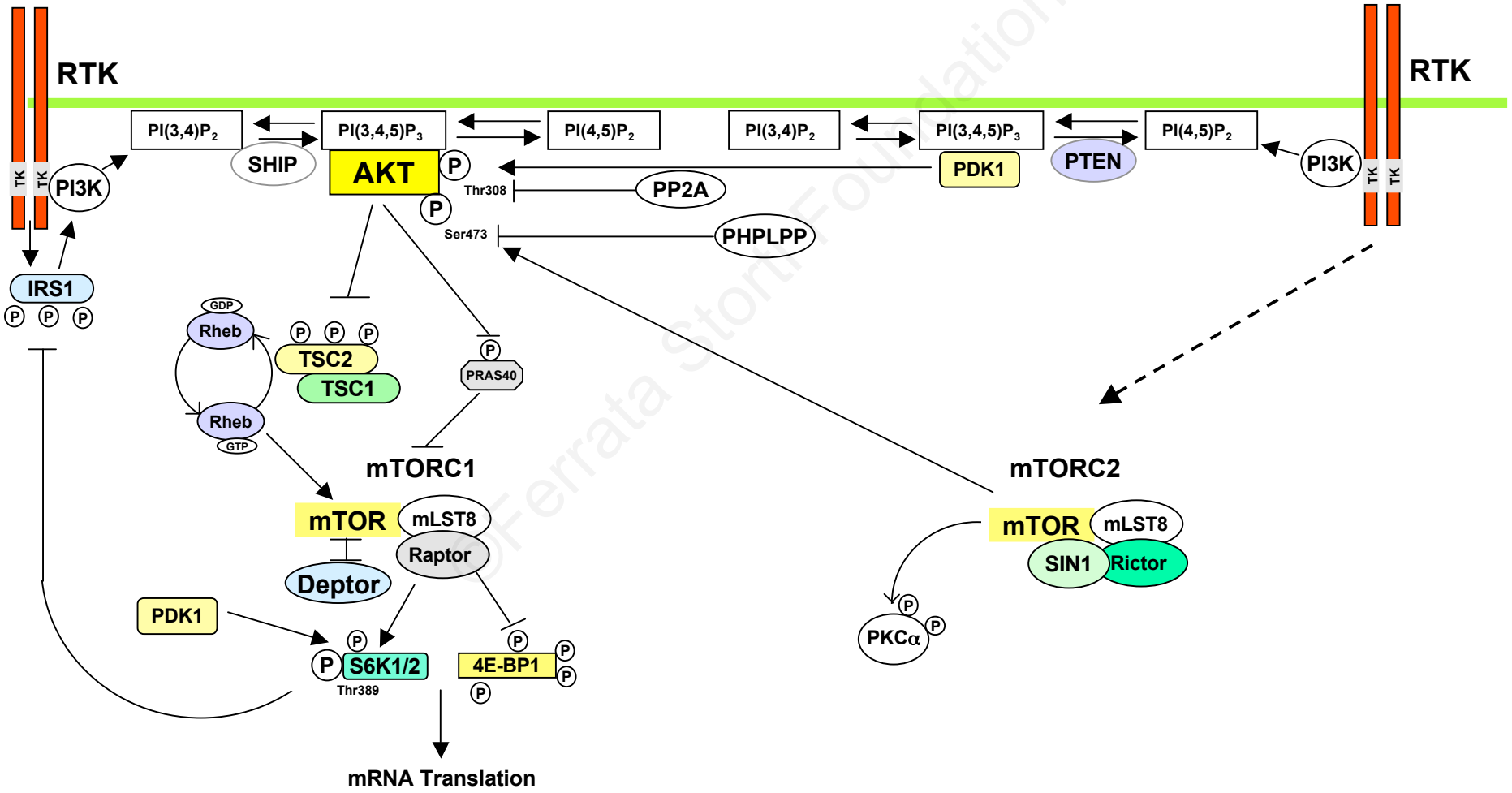
DOI: 10.3324/haematol.2009.013797

Figure 1



DOI: 10.3324/haematol.2009.013797

Figure 2



DOI: 10.3324/haematol.2009.013797

Table 1: Mechanisms potentially contributing to PI3K/AKT activation in AML

Molecules	Alteration
FLT3	Mutations
KIT	Mutations
RASN (N-Ras) and RASK (K-Ras)	Mutations
PK3CD (PI3K p110 subunit delta)	Unknown
mTORC2	Unknown
ILK	Unknown
PTEN	Phosphorylation/Decreased expression
SHIP	Mutations
ITA4 (VLA-4) / FINC (Fibronectin)	Blast cell interactions with the stroma
IGF-1/IGF1R	Autocriny
VEGF/VEGF-R	Autocriny/Paracriny
Angiopoietin	Autocriny/Paracriny

DOI: 10.3324/haematol.2009.013797

Table 2: Therapeutic targets in the PI3K/AKT and mTOR signalling pathways in AML

Molecular targets: specificity	Molecules	Clinical trials
PI3K and PIKK	Wortmanin (generic)	
	LY294002 (generic)	
PDK1	KRX0601 (Kyowa Hakko Kogyo)	
PK3CD (PI3K p110 subunit delta)	IC87114 (ICOS)	
	PCN12 (Piramed)	
	PCN118 (Piramed)	
mTORC1	Rapamycin/Sirolimus (Wyeth) (Rapamune)	phase II, NCT00634244
	Everolimus (Novartis) (RAD001/Afinitor)	phase I, NCT00544999, NCT00636922, phase II in association with AMN107 NCT00762632, phase I in association with PKC-412 NCT00819546
	Temsirolimus (Wyeth) (CCI-779/Torisel)	phase II, NCT00084916
	Deforolimus (Ariad) (AP23573)	phase II NCT00086125
mTOR (TORKinhib)	Ku-0063794 (AstraZeneca) (ref 93)	
	PP242 and PP30 (ref 92)	
PI3K and mTOR	PI-103 (ref 86,87)	
	NVPBEZ235(Novartis)	phase I in breast cancer NCT00620594
	BGT 226 (Novartis)	phase I-II solid tumor, breast cancer
	XL 765 (Exilixis)	phase I solid tumor, malignant gliomas
AKT	PIAs	
	Deguelin (generic)	
	Perifosine (AEterna Zentaris) (KRX-0401)	phase II, NCT00391560/ phase I, NCT00301938 in association with UCN-01
	Tricibine (VioQuest) (VDQ-002 or API-2)	phase I, NCT00642031
	KP372-1 (QLT)	
IGF-1/IGF1R	NVP-AEW541 (Novartis)	
	Anti-IGF-1 Ab: AVE-1642 (Sanofi-Aventis)	phase I NCT00791544 metastatic liver carcinoma
SHIP1	AQX-016A (Aquinox)	
	AQX-MN100 (Aquinox)	
Inhibitors of multiple kinases including PDPK1	7-hydroxystaurosporine (Kyowa Hakko Kogyo)	phase I, NCT00004263, NCT00301938
	BAG956 (Novartis)	
HSP90	Tanespimycin (Bristol-Myers Squibb)	phase II, NCT00514371 in multiple myeloma