

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

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## ABSTRACT

The PI3K/AKT and mTOR signaling pathways are activated in acute myeloid leukemia, including in the more immature leukemic populations. Constitutive PI3K activation is detectable in 50% of acute myeloid leukemia samples whereas mTORC1 is activated in all cases of this disease. In leukemic cells, the PI3K activity relates to the expression of the p110 $\delta$  isoform of class IA PI3K. Constitutive PI3K activation is the result of autocrine IGF-1/IGF-1R signaling in 70% of acute myeloid leukemia 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 acute myeloid leukemia, dual PI3K and mTOR inhibitors may induce apoptosis in blast cells. Moreover, mTORC1 inhibition using sirolimus

overactivates PI3K/AKT via the upregulation of IRS2 expression and by favoring IGF-1/IGF-1R autocrine signaling. Recent data also indicate that mTORC1 does not control protein translation in acute myeloid leukemia. These results open the way for the design of direct inhibitors of protein synthesis as novel acute myeloid leukemia therapies and also for the development of second generation mTOR inhibitors (the TORKinhibs).

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## Introduction

Acute myeloid leukemia (AML) comprises a group of clonal malignant diseases characterized by a deregulated proliferation of immature myeloid cells.<sup>1</sup> 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%.<sup>2</sup> 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 hematopoiesis.<sup>3</sup> Leukemic hematopoiesis shares similarities with normal hematopoiesis<sup>4,5</sup> 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.<sup>4,6</sup> In AML, deregulation of the signaling 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.<sup>7</sup> In this regard, the abnormal activation of PI3K/AKT, mTORC1, ERK/MAPK, STAT3/5, Wnt/ $\beta$ -catenin, and NF- $\kappa$ B has been

reported.<sup>8-20</sup> 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 signaling pathways and on recent data concerning their role, mechanisms of activation and interactions in AML biology.

## General biology of the class IA PI3K and mTOR signaling pathways

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

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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).

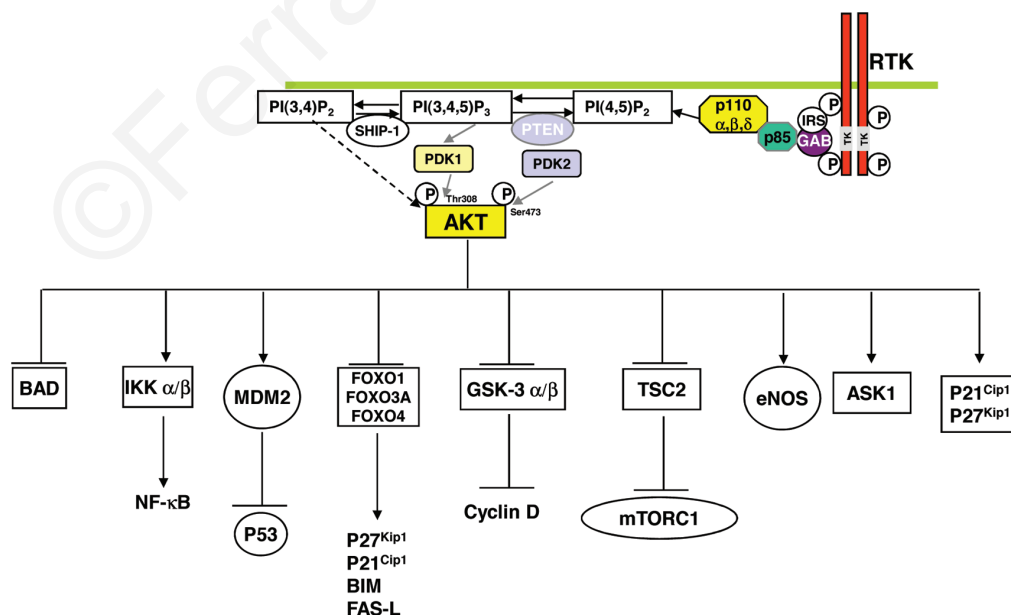
The AKT network controls different targets including the FOXO family of transcription factors. When they are unphosphorylated, the FOXOs (FOXO1, FOXO3A, FOXO4) localize in the nucleus and induce the transcription of a wide array of target genes involved in the cell cycle and apoptosis such as CDN1B (p27<sup>Kip1</sup>) and CDN1A (p21<sup>Cip1</sup>), Fas-L (TNFL6) and BIM.<sup>26</sup> PI3K activation downstream from growth factor receptors<sup>27</sup> 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.<sup>26</sup>

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.<sup>28</sup> Genetic inactivation of PTEN leads to constitutive activation of the PI3K/AKT/mTORC1 axis.<sup>29</sup> 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<sup>30</sup> (Figure 2). Moreover, it has been shown that the PML tumor suppressor inactivates phosphorylated AKT in the nucleus, i.e. PML specifically recruits AKT and its phosphatase PP2A to the nuclear bodies.<sup>31</sup>

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 homolog,<sup>32</sup> thereby allowing RHEB-GTP to activate mTORC1. The mTORC1 complex is composed of FRAP (mTOR), Raptor, PRAS40 and LSt8 (GβL)<sup>33</sup> 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.<sup>34,35</sup> 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.<sup>36</sup> MTORC1 activity is specifically inhibited by sirolimus (rapamycin, Wyeth), a drug that interacts with the FKBP1A (FKBP12) protein and binds the FRAP domain of the mTOR kinase.

The interactions between mTORC1 and PI3K are complex. MTORC1 activity negatively regulates PI3K.<sup>37</sup> 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<sup>37</sup> (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 up-regulate the expression of the IGF-1R at the protein level and also that mTORC1 plays a crucial role in this control.<sup>38</sup> 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.<sup>39</sup> MTORC2 also phosphorylates the serum and glucocorticoid protein kinase (SGK) and some isoforms of PKC.<sup>40,41</sup> 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.<sup>42</sup> Recently a new protein known as DEPTOR has been identified as an mTOR-interacting protein.<sup>43</sup> Taken together,



**Figure 1.** The PI3K/AKT signaling pathway. An activated tyrosine kinase receptor (RTK) recruits adaptors 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.



ing immature leukemic cells, but only when AKT is activated in the more differentiated blast cell population, suggesting that PI3K is activated at all stages of leukemic hematopoiesis.<sup>56</sup>

The mTORC1 pathway is also activated in AML.<sup>57-60</sup> 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.<sup>60</sup> The other target of mTORC1, the 4E-BP1 protein, is phosphorylated on Thr70 or on Ser65 in all primary AML samples.<sup>59,60</sup>

#### Mechanisms of PI3K/AKT and mTORC1 activation in acute myeloid leukemia

The mechanisms leading to PI3K/AKT activation in AML are not completely clear. PK3CD, the p110 $\delta$  isoform of class IA PI3K, is always expressed in AML cells, whereas the p110 $\alpha$  and p110 $\beta$  isoforms are heterogeneously expressed.<sup>9,61</sup> 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.<sup>9,61</sup> However, in blast cells stimulated with IGF-1, over-increased PI3K activity is also mediated by the p110 $\beta$  isoform recruited to the IGF-1 receptor after stimulation.<sup>62</sup> In acute promyelocytic leukemia (APL), class IA PI3K signaling is mediated by both the p110 $\beta$  and p110 $\delta$  isoforms.<sup>63</sup> 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.<sup>63</sup> It is known also that constitutive overexpression of wild-type PK3CD is sufficient to activate AKT and to transform cultured cells.<sup>64</sup> p110 $\delta$  is expressed at a high level in lymphocytes and myeloid cells<sup>65</sup> 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.<sup>65</sup> 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<sup>+</sup> and PI3K<sup>-</sup> AML samples and at similar levels to those found in normal CD34<sup>+</sup> cells (*our unpublished data*). This suggests that the overexpression of PK3CD is not responsible for PI3K/AKT activation. Activating mutations in the *PI3KCA* gene coding for the p110 $\alpha$  isoform have also been detected in different cancers.<sup>64</sup> However, no such mutation has been identified in primary AML blast cells.<sup>66</sup> Moreover, no activating mutation in the *PI3KCD* gene encoding p110 $\delta$  has yet been identified in a large cohort of PI3K<sup>+</sup> AML samples.<sup>67</sup> The constitutive activation of AKT1 has been reported in cancers and results from a mutation in its PH domain.<sup>68</sup> 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 signaling pathways and induces leukemia in mice.<sup>69</sup> No E17K PH mutation has yet been identified in AML.<sup>55,70</sup>

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.<sup>71</sup> Phosphorylation and the decreased expression of PTEN have been reported, but their significance remains controversial.<sup>72</sup> Lentiviral

**Table 1.** Mechanisms potentially contributing to PI3K/AKT activation in acute myeloid leukemia.

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

expression of the *INPP5D* gene encoding SHIP1 in CD34<sup>+</sup> cells from AML patients profoundly reduces their GM-CSF-induced proliferation.<sup>73</sup> 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<sup>+</sup> 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.<sup>10,67</sup> However, it has been shown that GAB2, which is over-expressed in AML, can mediate PI3K activation downstream of some mutant c-Kit receptors.<sup>74,75</sup>

The IGF-1/IGF-1R signaling pathway also activates PI3K in AML. Leukemic cells express functional IGF-1R and IGF-1 autocrine production is detected in AML blast cells.<sup>62,76,77</sup> Downregulation of the PK3CB (p110 $\beta$ ) and PK3CD isoforms of PI3K by RNA interference impairs IGF-1-stimulated AKT activation, cell growth and survival in AML cells.<sup>62</sup> 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.<sup>78</sup> 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.<sup>78</sup> Other potential mechanisms of PI3K activation in AML might also implicate autocrine/paracrine VEGF or angiopoietin secretion.<sup>79,80</sup> Blast cells also directly interact with the bone marrow micro-environment, and ITA4 (VLA-4) / FINC (fibronectin) interactions activate PI3K in blast cells.<sup>81</sup>

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.<sup>82</sup> 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 one 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.<sup>65</sup> 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 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;<sup>60</sup> b) IC87114 (ICOS)-induced PI3K/AKT specific inhibition does not suppress mTORC1 activity;<sup>77</sup> c) the Src kinase Lyn is constitutively phosphorylated in some leukemic cells and controls mTORC1 but not AKT activation;<sup>84</sup> 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.<sup>85</sup> Other unexplored signaling pathways may positively or negatively control mTORC1 activity in AML, including the amino acid pathway and the energetic LKB1/AMPK pathway.

*Functional role of PI3K/AKT and mTORC1 activation in acute myeloid leukemia*

The PI3K/AKT pathway controls blast cell proliferation and the clonogenicity of leukemic progenitors<sup>9,52</sup> and PI3K inhibition with LY294002 induces apoptosis in these progenitors.<sup>9</sup> 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.<sup>61</sup> 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.<sup>86</sup>

Rapalogs which belong to the first generation of allosteric mTORC1 inhibitors reduce blast cell proliferation and decrease the clonogenicity of leukemic progeni-

**Table 2. Therapeutic targets in the PI3K/AKT and mTOR signaling pathways in acute myeloid leukemia.**

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) Everolimus (Novartis) (RAD001/Afinitor)  Temsirolium (Wyeth) (CCI-779/Torisel) Deforolimus (Ariad) (AP23573)	phase II, NCT00634244 phase I, NCT00544999, NCT00636922, phase II in association with AMN107 NCT00762632, phase I in association with PKC-412 NCT00819546 phase II, NCT00084916 phase II NCT00086125
mTOR (TORKinhib)	Ku-0063794 (AstraZeneca) <sup>93</sup> PP242 and PP30 <sup>92</sup>	
PI3K and mTOR	PI-103 <sup>86,87</sup> NVPBEZ235 (Novartis) BGT 226 (Novartis) XL 765 (Exelixis)	phase I in breast cancer NCT00620594 phase I-II solid tumor, breast cancer phase I solid tumor, malignant gliomas
AKT	PIAs Deguelin (generic) Perifosine (AEterna Zentaris) (KRX-0401)  Tricibine (VioQuest) (VDQ-002 or API-2) KP372-1 (QLT)	phase II, NCT00391560/ phase I, NCT00301938 in association with UCN-01 phase I, NCT00642031
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 PDK1	7-hydroxystaurosporine (Kyowa Hakko Kogyo) BAG956 (Novartis)	phase I, NCT00004263, NCT00301938
HSP90	Tanespimycin (Bristol-Myers Squibb)	phase II, NCT00514371 in multiple myeloma

tor cells.<sup>57-60</sup> Surprisingly, these compounds are mainly cytostatic, as long-term treatment induces only very low rates of apoptosis.<sup>57-60</sup> However, inhibition of mTORC1 with everolimus has synergistic effects with cytosine arabinoside and etoposide.<sup>59</sup> 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 by increasing IRS2 expression in primary blast cells, thus promoting an autocrine IGF-1/IGF1R activation loop.<sup>77</sup> These data provide a rationale for simultaneously inhibiting both PI3K and mTORC1.<sup>77</sup> This was further supported by the demonstration that PI-103,<sup>87</sup> 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.<sup>88,89</sup>

We recently reported that protein synthesis is rapamycin-resistant in primary AML cells.<sup>60</sup> 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.<sup>60</sup> 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.<sup>90</sup> 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.<sup>60</sup>

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.

#### Prognostic role of PI3K/AKT activation in acute myeloid leukemia

An association between AKT phosphorylation and the clinical and biological characteristics of AML has yet to be detected.<sup>10</sup> Two studies have shown that PI3K<sup>+</sup> patients have a poorer prognosis than PI3K<sup>-</sup> patients.<sup>53,91</sup> 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.<sup>10</sup> The reasons for these different findings are not yet clear. One hypothesis for the lower relapse rate of PI3K<sup>+</sup> 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.<sup>55</sup>

#### 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.

#### 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.<sup>9,61</sup> 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<sup>+</sup> cells, and mice lacking PK3CD catalytic activity are viable and manifest defects in B cells, T cells and mast cells.<sup>92,93</sup> More potent inhibitors are currently under development by several drug companies such as Piramed, Novartis, and Calistoga. CAL-101 (Calistoga) has just entered Phase I trials for selected relapsed/refractory hematologic malignancies including AML [NCT00710528].

#### mTORC1 inhibitors

Treatment of PTEN-deficient mice with sirolimus restores normal stem cell function and blocks leukemogenesis.<sup>44</sup> This suggests that the inhibition of mTORC1 could be used to specifically target LSCs and not affect normal HSCs. Sirolimus and a number of analogs (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 4 out of 9 patients with either refractory or relapsed AML.<sup>57</sup> 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).<sup>94,95</sup> 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.<sup>94,95</sup>

#### Rationale for inhibiting both PI3K/AKT and mTORC1 as an acute myeloid leukemia 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.<sup>77</sup> In addition, the concomitant inhibition of PI3K and mTORC1 with IC87114 and everolimus, respectively, has additive anti-proliferative effects against AML *in vitro*.<sup>77</sup> However, the combination of these compounds is cytostatic and does not induce apoptosis.<sup>88</sup> Hence, novel inhibitors of both pathways, such as PI-103, appear to be promising.<sup>87,89</sup> As outlined above, PI-

103 inhibits blast cell proliferation and induces mitochondrial apoptosis in the LSC compartment<sup>88</sup> and not in normal CD34<sup>+</sup> cells. However, the bioavailability of PI-103 is not optimal. New dual inhibitors of PI3K and mTOR, such as NVP-BEZ235 (Novartis)<sup>96,97</sup> 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 easy 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.

#### **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<sup>98</sup> 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.<sup>98-100</sup>

#### **AKT inhibitors**

AKT inhibitors include the family of phosphatidylinositol ether analogs (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<sup>101</sup> but they can also activate p38MAPK and AMPK.<sup>102,103</sup> Perifosine (Aeterna, Zentaris) is an oral bioactive novel alkylphospholipid that dephosphorylates AKT 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<sup>+</sup> cells, and increases blast cell sensitivity to etoposide.<sup>104</sup> In blast cells, perifosine may increase the response of tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) cytotoxicity via the upregulation of TRAIL-R2 receptor expression and suppression of cFLIP expression.<sup>105</sup> 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.<sup>106</sup> 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].

#### **SHIP1 agonists**

Activation of the phosphatases that control PIP3 degradation might also inhibit PI3K. The meroterpenoid Pelorol and two structural analogs, AQX-016A (Aquinox) and

AQX-MN100 (Aquinox) exhibit SHIP1 activating activity in macrophages and mast cells.<sup>107</sup> As SHIP1 expression is restricted to the hematopoietic cells, these inhibitors might also specifically increase the activity of PI3K inhibitors in the hematopoietic system.

#### **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.<sup>76</sup> NVP-AEW541 induces apoptosis and sensitizes blast cells to etoposide treatment,<sup>62, 76</sup> 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. Inhibition of IGF-1R could be effective and tolerated for short-term periods but is most likely not suitable for maintenance therapy.

#### **Multiple kinase inhibitors**

The use of inhibitors targeting different signaling 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 cell apoptosis and inhibits the colony-forming ability of AML samples.<sup>108</sup> BAG956 (Novartis) is the first dual PI3K/PDK1 inhibitor that enhances the inhibitory effects of midostaurin (Novartis), an inhibitor of mutant FLT3<sup>109</sup> that is currently in Phase III trials for AML [NCT00651261]. BAG956 also has synergistic effects with rapamycin in different AML cell lines.<sup>109</sup>

#### **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.<sup>110</sup> The HSP90 inhibitor tanespimycin (Bristol-Myers Squibb) might, therefore, be useful in the treatment of PI3K<sup>+</sup> AML, in part through the destabilization of other client kinases, including FLT3, RAF and AKT.<sup>111,112</sup>

## **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.

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 signaling 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 signaling network that comprises a multitude of regulatory loops,<sup>113</sup> and the existence of the hematopoietic niche providing an external environment, which could influence the fate and the signaling pathways of AML blasts.<sup>114</sup> Finally, the con-

cept of the leukemic stem cell receiving important signals from the hematopoietic niche, that support self-renewal, could explain the chemoresistance found in this disease.<sup>115</sup> 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.

## 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.

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