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Spotlight

Therapeutic potential of inhibiting the PI3Kγ complex for leukemia

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In their recent paper published in *Nature*, Luo et al.[1] investigate the cancer-cell-intrinsic roles of the PI3Kγ complex in leukemia. Their findings pinpoint PI3Kγ inhibition as a possible novel treatment avenue for a subset of acute leukemias.

Aberrant activation of phosphoinositide 3-kinase (PI3K) signaling is one of the most common oncogenic events across cancer types. Humans have four PI3K catalytic subunits: (p110) α, β, γ, and δ (encoded by genes *PIK3CA*, *PIK3CB*, *PIK3CG*, and *PIK3CD*). PI3Kα and β are expressed in all cell types, whereas PI3Kγ and PI3Kδ are highly expressed in myeloid and lymphoid cells, respectively[2] (Figure 1A). PI3K catalyzes the production of the lipid phosphatidylinositol-3,4,5-triphosphate (PIP_3) and primarily exerts its effects in cancer cells through the AKT-mTOR pathway, which is well characterized. There are also PI3K-dependent and AKT-independent responses, such as PI3K-mediated activation of the glycolytic enzyme aldolase,[3] but these phenotypes are less well explored. Class IA PI3Ks (α, β, and δ) are regulated by the binding of receptor tyrosine kinases and small GTPases including Ras, which stimulate activity and signaling, whereas class IB PI3K (γ) is regulated by G-protein-coupled receptors and small GTPases. *PIK3CA* is one of the most frequently mutated oncogenes in cancer, but the other PI3K catalytic subunits are rarely mutated in cancer.

Over the past decades, scores of inhibitors targeting class I PI3Ks have been tested in multiple cancer types. These drugs had high expectations but have largely under-delivered due to low rates of efficacy and high rates of adverse events, notably on-target hyperglycemia due to inhibition of wild-type PI3Kα. Currently, the PI3Kα inhibitor alpelisib is approved in combination with anti-estrogen therapy for the treatment of *PIK3CA*-mutant, hormone-receptor-positive advanced breast cancer. It is notable that even in breast cancer, PI3K-inhibitor monotherapy is associated with low response rates and requires combination therapy for full clinical benefit. Multiple PI3Kδ inhibitors were initially approved for the treatment of low-grade lymphomas and chronic lymphocytic leukemia (CLL). However, given difficulties in accruing confirmatory trials, increased immune side effects, and a possible decrement in overall survival, many of these agents or indications have been withdrawn or removed from approval. Currently only duvelisib and idelalisib in combination with rituximab are approved PI3Kδ inhibitors in relapsed or refractory CLL. PI3Kγ inhibition has been shown to decrease solid tumor growth and increase the recruitment of cytotoxic T cells to the tumors.[4,5] The PI3Kγ-selective inhibitor eganelisib (IPI-549) is being tested in phase 1 and 2 clinical trials in advanced solid tumors, with first results published last year (NCT02637531).[6] These data showed that eganelisib in combination with the anti-PD-1 nivolumab demonstrates a low response rate as monotherapy (5–10%) corresponding to a 2- to 4-month improvement in progression-free survival, including in patients who had progressed on anti-PD-1/PD-L1, with minimal toxicities. Thus, decades of failed drug discovery punctuated by small gains have established that PI3K is not an Achilles heel but a more subtle vulnerability, with complex benefit-risk calculations due to competing tensions of efficacy, toxicity, and biomarkers. Therefore, moving PI3K inhibitors forward in the clinic requires the discovery of new PI3K-inhibitor combination therapies in specific biomarker-driven subsets of cancer.

Acute leukemias are rapidly progressing cancers that derive from different blood cell lineages. Conventional induction chemotherapy is the standard of care in acute leukemias, with some targeted therapies approved in patients whose tumors exhibit the appropriate genomic biomarker (e.g., PML-RARA fusion, BCR-ABL fusion, or IDH1/2 mutations). Blastic plasmacytoid dendritic cell neoplasm (BPDCN) is a rare acute myeloid malignancy commonly affecting older men who often present with skin lesions. BPDCN derives from type 1 interferon producing cells and bears a CD123+ immunophenotype. The CD123-directed immunotoxin tagraxofusp is the first approved therapy for the treatment of BPDCN; it confers a ∼50% complete response rate and a ∼12-month duration of complete response, which is much higher than standard historical induction chemotherapies.[7] The 5-year overall survival rates for leukemia vary from 90% in acute promyelocytic leukemia (APL) to 65% in B-cell acute lymphocytic leukemia (ALL) to 15% in acute myeloid leukemia (AML). It is critical to discover new targeted therapies to improve survival in acute leukemia.

Through their investigations of novel gene dependencies in leukemias, Luo et al.[1] have identified a subset of acute leukemias that depend on PI3Kγ signaling. Using CRISPR interference screens in BPDCN cells, they discovered that targeting *PIK3R5*, which encodes the p101 regulatory subunit of the PI3Kγ complex, decreased cell growth. This effect was

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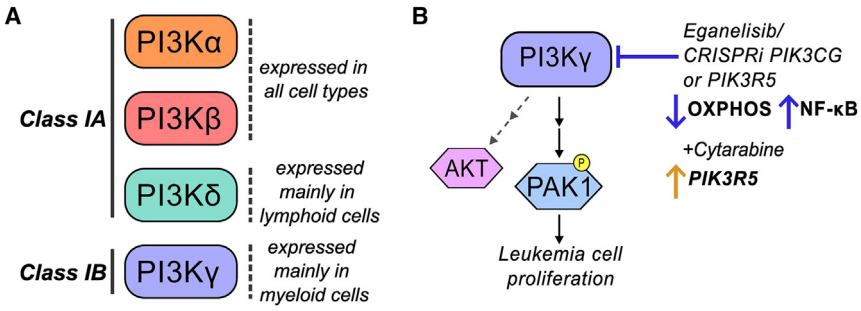

Figure 1. PI3Kγ complex identified as a leukemia dependency
A. Isoforms and cell-type specificities of class I PI3K complexes.
B. Summary of PI3Kγ dependency and its AKT-independent downstream effects in a subset of acute leukemias described by Luo et al.[1]

specific to PI3Kγ, and targeting the catalytic or regulatory subunits of other PI3K isoforms was not associated with BPDCN lethality. Whereas *PIK3R5* elevation appears to be the most dominant genomic feature of the leukemia subtypes characterized in this paper, selected leukemia cell lines (AML and BPDCN) responded to knockdown of both *PIK3R5* and *PIK3CG* specifically in cell lines with high *PIK3R5* or *PIK3CG* expression. Treatment with the PI3Kγ-selective inhibitor eganelisib also recapitulated this phenotype, inducing apoptosis and myeloid differentiation.

Further analysis showed that in PI3Kγ-dependent leukemias across different lineages, increased innate immune signaling through Toll-like receptors (TLR) is associated with high levels of *PIK3R5* expression. Mechanistically, PIK3R5 transcription is mediated by the SPI1 transcription factor, which binds to the *PIK3R5* promoter under TLR stimulation. The protein levels of *PIK3R5* and *PIK3CG* were also decreased when the other was depleted, whereas mRNA levels were not affected, consistent with a model in which p101 and p110γ proteins protect each other from turnover. Notably, this is distinct from PI3Kα, where p85α (the regulatory subunit of PI3Kα, encoded by *PIK3R1*) is in excess in many cell types, forming not only PI3Kα complexes where p85α both stabilizes and inhibits p110α catalytic activity but also additional "p110α-free" p85α, which can bind to receptor tyrosine kinases (RTK) and prevent PI3Kα binding. Thus, *PIK3R1* is a tumor suppressor whose loss, in the form of decreased p110α-free p85α, leads to increased binding of PI3Kα to RTKs for activation.[8] These differences highlight distinctions in cell-specific regulation of PI3K isoforms.

The authors then elucidated the downstream consequences of PI3Kγ inhibition in leukemia. They showed that eganelisib treatment or *PIK3R5* knockdown in acute leukemia cells suppressed oxidative phosphorylation and activated NF-κB signaling (Figure 1B). Surprisingly, neither the genetic nor the pharmacologic inhibition of PI3Kγ in leukemia cells was associated with decreased AKT signaling, suggesting a non-AKT-dependent mechanism. Further investigation into the downstream effectors of PI3Kγ implicated p21-activated kinase 1 (PAK1) and its phosphorylation at S144, which is downregulated by PI3Kγ inhibition. PI3Kγ inhibition was also associated with decreased binding of PAK1 to the small GTPase RAC1, which activates PAK1. Moreover, lack of PAK1 S144 phosphorylation correlated with PI3Kγ inhibitor resistance in patient-derived leukemia cells. In further support of PAK1 as a key node downstream of PI3Kγ, PAK1 kinase inhibitors mimicked the effects of eganelisib while AKT kinase inhibitors did not, and a PAK1 S144D phosphomimetic mutation rescued the effects of eganelisib.

Finally, the authors tested the translational potential of eganelisib treatment in combination with cytarabine, a common cytotoxic chemotherapy in leukemia, in xenograft mice models. The combination treatment with eganelisib and cytarabine increased survival of mice compared to single agent treatments in BPDCN, AML, and ALL cells with elevated *PIK3R5* levels. Interestingly, combination treatment also increased survival in *PIK3R5*-low BPDCN, AML, and ALL cells, in which eganelisib alone did not affect survival. This effect appears to be due to a cytarabine-induced increase in *PIK3R5* expression, which created a PI3Kγ signaling dependency sensitive to eganelisib treatment. This indicates potential for the wider usage of eganelisib in combinatorial treatments.

Together, these data provide strong support for a PI3Kγ-dependent vulnerability across *PIK3R5*-expressing leukemias of different lineages through an AKT-independent mechanism that is dependent on PAK1. PI3Kγ inhibition in these leukemias leads to decreased cell growth, increased myeloid differentiation, decreased oxidative phosphorylation, and increased NF-κB signaling. This work adds to the small but growing literature of PI3K-dependent, AKT-independent phenotypes important in physiology and for predicting therapeutic responses.

One key question remains: what is the mechanism connecting PI3Kγ with PAK1 and RAC1? A strong contender for this role is one of >20 guanine nucleotide exchange factors (GEFs) for RAC1.[9] Most RAC1 GEFs contain PH domains that bind to PIP_3 in cell membranes and are therefore dependent on PI3K. They can also activate RAC1 through direct binding and extrusion of GDP; this allows the binding of GTP, which is at high concentrations in the cell. Interestingly, one of these RAC1 GEFs, *ARHGEF6* (αPIX), can associate with the PI3K regulatory subunit and promote PAK1 activation.[10]

Given the favorable preclinical efficacy of eganelisib and its favorable toxicity profile in the clinic, PI3Kγ inhibition appears as a bright new target for leukemia therapy that is ripe for clinical investigation. It will be important to determine whether high *PIK3R5* expression levels are necessary for clinical response in patients and whether decreased phosphorylated PAK1 may serve as an *in vivo* pharmacologic biomarker. Moreover, it will be critical to understand whether PI3Kγ inhibition is effective after standard therapies including tagraxofusp in BPDCN, whether other combination therapies result in synergistic tumor response, and the biological basis of therapy resistance. In sum, this work by Luo et al.[1] serves as a model for investigating isoform-specific PI3K inhibition in a rare cancer to illuminate therapeutic vulnerabilities across an entire cancer lineage.

DECLARATION OF INTERESTS

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