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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

Defining a Targetable Leukemia Intrinsic Dependency on Noncanonical PI3Kgamma Signaling

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There are overlapping clinical and pathologic characteristics between poor prognosis subsets of acute myeloid leukemia (AML) and blastic plasmacytoid dendritic cell neoplasm (BPDCN), but common dependencies are ill-defined. We hypothesized that identifying novel shared targets and biomarkers could elucidate biology and nominate new therapies for both malignancies. We performed genome-wide CRISPR interference in BPDCN cells and identified 327 dependency genes, which were then analyzed informatically and functionally in AML, ALL, and BPDCN cells and patient-derived xenografts (PDXs). We defined a leukemia subset characterized by *PIK3R5* activation and sensitivity to phosphoinositide 3-kinase gamma (PI3Kγ) inhibition via a noncanonical downstream pathway.

The PI3Kγ complex includes an enzymatic subunit p110γ encoded by *PIK3CG* and a regulatory subunit p101 encoded by *PIK3R5*. Intriguingly, despite elevation of both p110γ and p101 proteins in this leukemia subset, only the *PIK3R5* mRNA is overexpressed. To investigate this disconnection, we measured protein half-life and found that p110γ stability is positively regulated by *PIK3R5*/p101, associated with reduced p110γ ubiquitination. This suggested that increased expression of *PIK3R5*/p101 leads to posttranslational p110γ elevation by protecting it from ubiquitin proteasome-mediated degradation.

To identify the upstream activator of *PIK3R5*, we analyzed RNA-seq data in >200 leukemia PDXs and found that AMLs with activated *PIK3R5* are enriched for an innate immune response signature (IIRS). Supporting this model and indicating potential clinical relevance, analysis of TCGA AML patients revealed that the IIRS, which includes *PIK3R5*, was associated with M4/M5 monocytic AML and poor prognosis, independent of age, cytogenetics, or somatic mutations. Furthermore, in cells with low baseline expression, *PIK3R5* was induced by the Toll-like receptor agonist resiquimod, showing that *PIK3R5* is activated by inflammatory signaling and consistent with a monocytic/dendritic lineage association. By integrated analysis of ATAC-seq and ChIP-seq in PIK3R5 high vs low leukemias, as well as gene dependency scores, we identified the transcription factor PU.1 as responsible for *PIK3R5* activation. Treatment with resiquimod enhanced the interaction between PU.1 and the *PIK3R5* promoter, while depletion of PU.1 nullified the ability of resiquimod to activate *PIK3R5*.

To elucidate molecular events downstream of p101/PI3Kγ, we studied consequences of pathway inactivation using *PIK3R5* interference or the PI3Kγ-selective inhibitor eganelisib. Surprisingly, there was no effect of PI3Kγ inactivation on AKT phosphorylation or canonical PI3K-AKT-mTOR mRNA targets. Therefore, we performed phosphoproteomics and found that PAK1 S144, a phosphorylation site not previously associated with PI3K signaling, was suppressed by PIK3R5/PI3Kγ inhibition (**panel a**). Selective inhibitors of PAK1, but not AKT, replicated the effect of eganelisib. Furthermore, introduction of constitutively active PAK1, but not inactive PAK1, eliminated sensitivity of leukemias to eganelisib, supporting the functional relationship between PAK1 phosphorylation and PI3Kγ dependency.

PI3Kγ inhibitors are being tested in solid tumor trials to repolarize macrophages and potentiate immune checkpoint blockade, but their cancer cell-intrinsic role has not been explored. To assess PI3Kγ inhibition in vivo, we first performed intradermal xenografting assays using BPDCN derived from a patient's skin tumor. Eganelisib achieved comparable efficacy to cytarabine chemotherapy and the combination of eganelisib and cytarabine exerted significant synergy (combination index=0.63, $p<0.0001$). Next, we measured the effect of PI3Kγ inhibition on survival in disseminated PDX models. As predicted, single agent eganelisib significantly prolonged survival in AML, ALL, and BPDCN with high PIK3R5, while having no benefit in cases with low PIK3R5. To our surprise, the combination of eganelisib/cytarabine synergistically increased survival in all cases, regardless of baseline PIK3R5 (**panel b**). We conclude that inflammatory signaling-activated PIK3R5 is a biomarker for sensitivity to PI3Kγ inhibition via an unappreciated PI3Kγ-PAK1 axis. Synergy with cytarabine unmasked eganelisib sensitivity in additional leukemias, expanding the therapeutic potential of targeting PI3Kγ.

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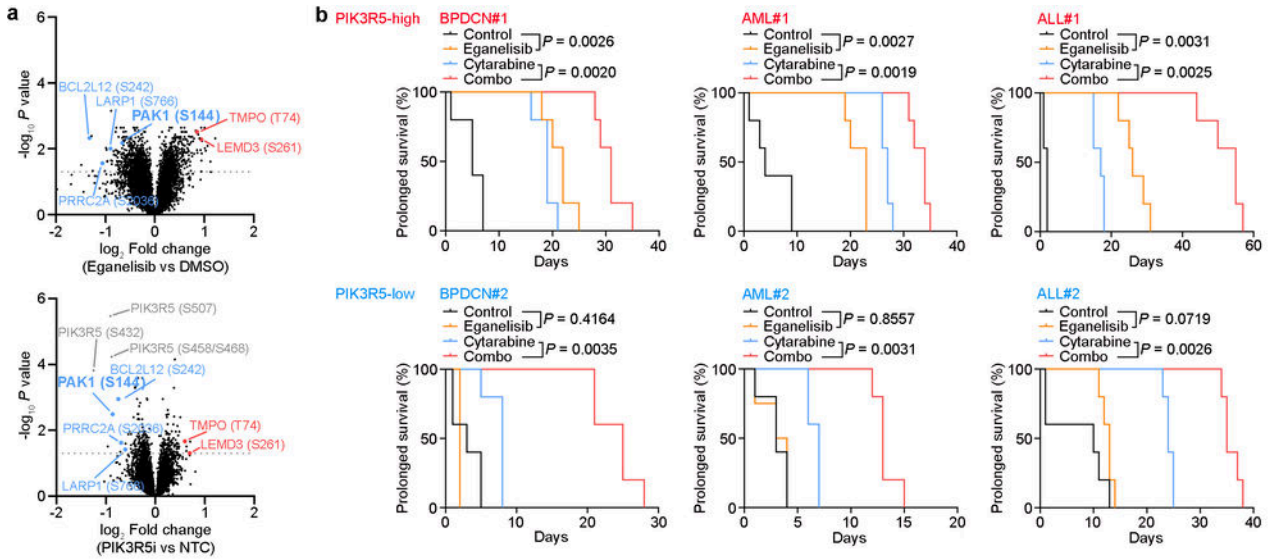

Targetable dependency on PI3Kγ/PAK1 signaling in leukemias with PIK3R5 activation. a, Volcano plots showing consistently downregulated (blue) or upregulated (red) phosphorylated peptides upon PI3Kγ inhibitor eganelisib treatment and PIK3R5 CRISPR interference (PIK3R5i) from mass spectrometry-based phosphoproteomics. PAK1 S144 was confirmed as the essential phosphorylation site that contributes to the dependency of leukemias on PI3Kγ in follow-up mechanistic and functional studies. **b,** Prolonged survival in leukemia PDXs with intrinsic high (red) or low (blue) PIK3R5 expression upon treatment with eganelisib and/or cytarabine.

Figure 1

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