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Resolving PI3K-δ inhibitor resistance in CLL

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Summary:

In this issue of *Blood*, Scheffold and Chelliah Jebaraj et al identify enhanced signalling through IGF1R as the resistance mechanism of CLL cells to a PI3K-δ inhibitor in a CLL animal model. They also show that the inhibition of IGF1R provides a salvage treatment for PI3K-δ inhibitor-resistant tumors.

Main text:

Recent successes in cancer treatment by targeted therapies are partly undermined by the development of resistance to the therapies. Therefore, it is imperative to unravel the underlying molecular resistance mechanisms in order to develop salvage therapies. Specific targeting of pathways comprising PI3K-signalling has revolutionized the treatment of chronic lymphocytic leukemia (CLL) and provides a paradigm of targeted therapy for the mode-of-action of pathway-specific drugs and for the identification of resistance mechanisms.[1-3] Drugs inhibiting two components of the PI3K-pathway, ibrutinib (targeting Bruton's-tyrosine kinase; BTK) and idelalisib (targeting phosphoinositide-3-kinase-δ; PI3K-δ) are highly efficacious in the treatment of CLL and other non-Hodgkin lymphomas (Fig.1A). However, whereas recent studies have uncovered causes of ibrutinib-resistance,[4,5] the mechanism of resistance to idelalisib has remained elusive.

Scheffold et al addressed this deficiency by modelling resistance to PI3K-δ inhibitors *in vivo* in two murine serial adoptive-transfer and treatment models.[6] First, CLL cells derived from the commonly used Eμ-TCL1 CLL-model were transplanted into syngeneic mice, thus ensuring the presence of an intact tumor-microenvironment. Second, an Eμ-TCL1 CLL-derived, well-characterized cell line (TCL1-192)[7] was transplanted into NOD/SCID mice. The authors observed in both models that mice treated with PI3K-δ-inhibitor, while initially

benefiting from PI3K-δ-inhibition, eventually succumbed to the disease. Scheffold and colleagues then used the experimentally more amenable TCL1-192 line to develop a serial-transfer and treatment scheme to continuously treat CLL *in vivo*, thus generating tumors resistant to PI3K-δ-inhibition that were then subjected to whole-exome sequencing (WES).

Naturally, the expectation was to identify mutations in PI3K-δ that abolish the action of the inhibitor, or recurrent activating genetic alterations in pathway genes downstream of PI3K-δ. However, the WES-analysis did not identify any single recurrent mutation in PI3K-δ,[6] which was particularly surprising since mutations in BTK at the inhibitor-binding site are the hallmark of ibrutinib-resistant CLL.[4,5] Also, no recurrent mutations were found in other genes. Similarly, CLL cells from patients that progressed under idelalisib-treatment were found to be devoid of unifying recurrent mutations that could explain drug resistance.[8]

Scheffold et al then transcriptionally profiled these tumors and identified several genes whose expression was deregulated in PI3K-δ inhibitor-resistant vs. sensitive tumors.[6] Upregulated expression of insulin-like growth factor-1 receptor (IGF1R) showed the strongest association with resistance. They then demonstrated that IGF1R-upregulation indeed contributed to PI3K-δ-inhibitor resistance (Fig.1B). But, how is IGF1R-upregulation in the resistant cells mediated?

Following a report showing that *IGF1R* can be transcriptionally activated by FOXO1 and GSK3,[9] the authors investigated their potential role in PI3K-δ-inhibitor resistance.[6] Indeed, the upregulation of *IGF1R*-expression was found to be attenuated by inhibition of GSK3 or FOXO1, indicating that enhanced IGF1R-expression upon PI3K-δ-inhibitor resistance is at least partially mediated by GSK3-activity that causes increased nuclear localization of FOXO1. Furthermore, the authors provided evidence that IGF1R promotes tumor-cell growth through activation of the MAPK-pathway.

Next, Scheffold and colleagues investigated whether PI3K-δ-inhibitor resistance can be overcome by pharmacological inhibition of IGF1R with linsitinib.[6] They demonstrated in well-

conceived *in vitro* and *in vivo* experiments that the combination of linsitinib with PI3K-δ-inhibitors, but not linsitinib-only treatment, was toxic for PI3K-δ-inhibitor-resistant CLL cells (Fig.1C). Linsitinib-only treatment did not impair tumor-cell growth as IGF1R-expression was downregulated in PI3K-δ inhibitor-resistant cells in the absence of PI3K-δ-inhibitor. This downregulation is likely due to reactivation of PI3K/AKT-signaling which inhibits nuclear translocation of FOXO1. Conversely, in the combination treatment, PI3K/AKT-inhibition leads to GSK3 and FOXO1-activation that enhances expression of IGF1R-protein which is then targeted by linsitinib. Overall, the results indicate that IGF1R-upregulation promotes cell growth of PI3K-δ inhibitor-resistant CLL cells via activation of the MAPK-pathway, and consequently that PI3K-δ-inhibitor resistance could be overcome by simultaneous PI3K-δ/IGF1R-inhibition.

Now, what is the translational aspect? Ideally, one would similarly analyze idelalisib-resistant CLL cells from patients enrolled in an ongoing trial. This could conclusively validate—or refute—the clinical relevance of the findings. This major undertaking is the authors' future aim. However, they may be on the right track: In one patient's CLL-sample with high IGF1R-expression after 10-months of idelalisib-treatment, the authors could demonstrate *in vitro* that those cells showed a low response to PI3K-δ-inhibitor and that idelalisib/linsitinib-combination treatment synergistically impaired cell growth, indicating that co-inhibition of IGF1R can sensitise tumor cells with reduced sensitivity to PI3K-δ inhibitors.[6] Another finding may be equally relevant: Some untreated CLL patients showed high *IGF1R*-expression in the tumor cells, particularly in association with trisomy-12. Also those patients may benefit from idelalisib/linsitinib-combination treatment.

The present work highlights the importance of animal models in the identification of drug-resistance mechanisms. Although it remains to be determined to what extent the newly identified IGF1R-mediated PI3K-δ-inhibitor resistance mechanism plays a role in idelalisib-resistance in human CLL, the results provide a clear indication of what to keep an eye out for in clinical trials. The work also emphasizes that resistance mechanisms may not be

restricted solely to genetic mutations. However, one caveat of modelling drug resistance in mice is the much shorter experimental time-span of disease progression compared to humans and the impact this may have on clonal evolution of the tumor. This could be particularly relevant in the case of PI3K-δ-inhibition, which was shown to increase genomic instability in B-cells and may thus introduce mutations by enhanced activity of activation-induced cytidine deaminase, which normally initiates somatic hypermutation and class switching, in off-target genes.[10] Since idelalisib can be administered for a very long time, there is the potential to uncover additional resistance mechanisms that involve genetic alterations which activate tumor-promoting pathways. In this context, it will be interesting to integrate the findings of the genomic analyses from mice[6] and humans[8] as this may uncover pathways that contribute to PI3K-δ-inhibitor resistance in some CLL-cases. Clearly, this excellently conducted work has provided a wealth of information and intriguing new leads that will be instrumental in overcoming the problem of idelalisib-resistance in human CLL and other malignancies. In the short term, the primary focus should be on determining whether linsitinib turns out to be the ideal companion of idelalisib in the treatment of lymphoid tumors with IGF1R-upregulation due to PI3K-δ-inhibitor resistance.

Conflict-of-interest disclosure: The author declares no competing financial interests.

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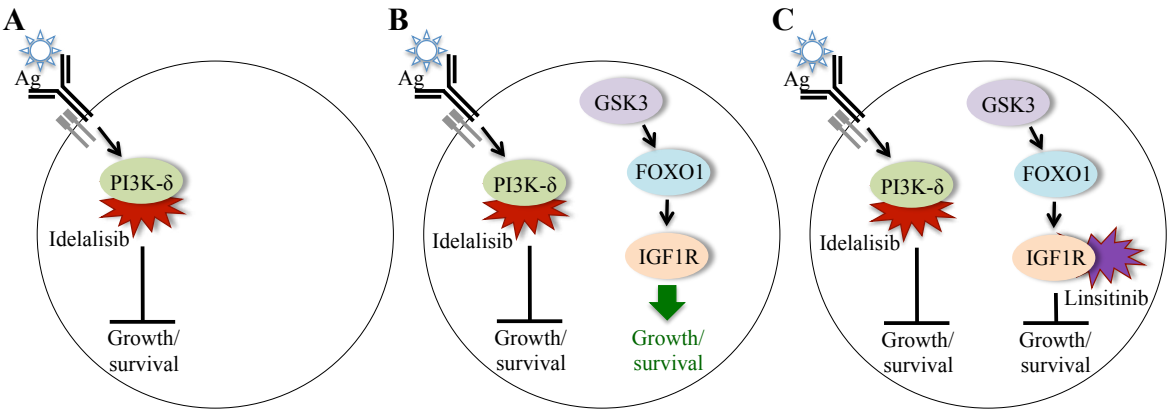
FIGURE LEGEND

Figure 1: Model for the effects of the PI3K-δ-inhibitor idelalisib and the IGF1R-inhibitor linsitinib on PI3K-δ inhibitor-sensitive and resistant CLL cells. (A) PI3K-δ is activated by the B-cell receptor (Ag, antigen) and inhibited by idealisib, thus ablating the PI3K pathway-mediated growth and survival program that activates AKT-signaling which is required for CLL cell survival. (B) Upregulation of IGF1R-expression via GSK3 and FOXO1 activates the MAPK pathway-mediated growth and survival program, thus leading to CLL cell survival. It is not known what leads to the activation of GSK3 and FOXO1 in PI3K-δ inhibitor-resistant CLL cells; however, FOXO1-activation is normally inhibited by AKT-signaling downstream of the PI3K-pathway. (C) Inhibition of IGF1R by linsitinib abolishes the MAPK-mediated growth and survival program, causing cell death in PI3K-δ-resistant CLL cells. The simultaneous inhibition of PI3K-δ is required since reactivation of PI3K/AKT-signaling leads to FOXO1-inhibition and failure to upregulate IGF1R. In the serial adoptive-transfer and treatment experiments, Scheffold et al used the specific PI3K-δ-inhibitor GS-649443 (rather than idelalisib) as this has favourable pharmacokinetic properties in mice.



CLL cell with PI3K-δ inhibition

PI3K-δ-inhibitor resistant CLL cell

PI3K-δ-inhibitor resistant CLL cell with PI3K-δ and IGF1R inhibition