inflammatory response, including neutrophil recruitment, in sepsis is a double-edged sword. Although inflammation is needed for pathogen clearance, it also mediates organ damage.\textsuperscript{6,7} Traditionally, \( \beta_2 \) integrins were considered critical players in neutrophil–endothelial interactions and transendothelial migration during inflammation, infection, and sepsis.\textsuperscript{8,9} However, the constitutive expression of \( \beta_2 \) integrins in leukocytes and the impaired pathogen clearance associated with \( \beta_2 \)-integrin inhibition, which has an adverse effect in survival, renders their blockade in the setting of microbial infection difficult.\textsuperscript{10}

Thus, the need for novel, specific biomarkers to diagnose and stratify septic patients is imperative. In addition, it is important to identify and therapeutically target pathways that specifically mediate neutrophil recruitment and activation in sepsis without having an impact on pathogen clearance.

Lerman et al identified \( \alpha_3 \beta_1 \) integrin as such a therapeutic target. They demonstrated that it is specifically overexpressed in neutrophils in sepsis and plays a role in the hyperresponsiveness of these cells.\textsuperscript{1} The expression of this integrin on neutrophils is highly upregulated in septic patients but not in patients with noninfectious inflammatory response syndrome; moreover, \( \alpha_3 \beta_1 \)-integrin expression was increased in neutrophils from mice subjected to experimental endotoxemia or cecal ligation and puncture–induced sepsis.\textsuperscript{3} These data suggest that neutrophil \( \alpha_3 \beta_1 \)-integrin expression could be a specific biomarker for sepsis. The increased \( \alpha_3 \beta_1 \) expression in a subpopulation of neutrophils correlated with a hyperactivated phenotype and associated with increased interleukin-6 production and myeloperoxidase activity. Strikingly, pharmacologic blockade of \( \alpha_3 \beta_1 \) integrin with the cyclic peptide LXY2 or neutrophil-specific inactivation of \( \alpha_3 \) not only abrogated neutrophil infiltration into the organs (eg, the lung) of septic mice, but also improved survival to septic shock (see figure) without affecting bacterial clearance.\textsuperscript{1} The decreased neutrophil extravasation resulting from \( \alpha_3 \beta_1 \)-integrin inhibition was associated with neutrophils entrapped in the space between the endothelial cells and pericytes, as shown by intravital and electron microscopy (see figure). These data make a strong case that the interaction between \( \alpha_3 \beta_1 \) integrin and its ligands in the extracellular matrix (eg, laminin) is critical for neutrophil migration beneath the endothelium and through the vascular wall. Additionally, \( \alpha_3 \beta_1 \) integrin was involved in neutrophil activation and interleukin-6 production following TLK2 ligation.\textsuperscript{3}

Taken together, the study by Lerman demonstrates a previously unrecognized role for \( \alpha_3 \beta_1 \) integrin in neutrophil infiltration and activation in response to pathogen-derived stimuli in the course of sepsis. The specific upregulation of \( \alpha_3 \beta_1 \) integrin on septic neutrophils may provide an important novel biomarker and diagnostic tool in sepsis, which merits further investigation in clinical settings. However, the implications of this study are over and above the diagnostic potential of neutrophil \( \alpha_3 \beta_1 \)-integrin expression. In particular, \( \alpha_3 \beta_1 \) blockade could represent an attractive novel therapeutic strategy to ameliorate systemic inflammation-induced organ damage and thereby improve survival in sepsis without having a negative impact on pathogen clearance. It is definitively worth planning further preclinical and clinical studies to assess targeting \( \alpha_3 \beta_1 \) integrin in the treatment of sepsis.

Conflict-of-interest disclosure: The authors declare no competing financial interest.

\textsuperscript{**LYMPHOID NEOPLASIA**}

Comment on Dong et al, page 3583

**PI3K in CLL: are 2 isoforms better than 1?**

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In this issue of \textit{Blood}, Dong et al present a series of experiments with the novel B-cell receptor (BCR) kinase inhibitor IPI-145 (phosphatidylinositol 3-kinase \( \gamma \)-/8 [PI3K\( \gamma \)/\( \delta \)]) inhibitor) and show that chronic lymphocytic leukemia (CLL) samples resistant to ibrutinib remain sensitive to killing by this agent.\textsuperscript{1}

In normal healthy B cells, the BCR detects and responds to antigens displayed on pathogens. Upon engagement, it activates 2 key processes; first, it triggers the formation of the "signalosome," a complex of kinases and proteins leading to downstream signal transduction consequent to apoptosis, proliferation, and/or migration (see figure); second, it internalizes the captured antigen for processing and subsequent presentation to T cells.

Strong evidence now exists to suggest that BCR signaling has a major role in regulating the behavior of CLL cells, and roles in the development, progression, and clinical response to treatment have all been proposed.\textsuperscript{2} These observations provide a strong rationale for targeting the BCR in CLL. BCR kinase inhibitors for the treatment of CLL have shown impressive clinical responses, which has led to significant therapeutic advances in the treatment of sepsis.

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difficult-to-treat and otherwise refractory patients. Two inhibitors of the BCR pathway have recently been approved by the Food and Drug Administration for various cohorts of CLL: the Bruton tyrosine kinase (BTK) inhibitor ibrutinib and the PI3K inhibitor idelalisib. Both drugs were hypothesized to exert their therapeutic effects by inhibiting CLL cells. However, the caveat is that samples that were resistant to ibrutinib were still susceptible to killing from IPI-145 even though BTK exerts its effect both upstream and downstream of PI3K, suggesting PI3K might compensate after BTK/phospholipase C-γ2 mutations/disruption. IPI-145 significantly inhibited signaling with anti-immunoglobulin M at much lower concentrations than idelalisib1,9; however, pAKT1/2T308 and ERK 1/2T202/Y204 were completely abrogated and confirm PI3K inhibition, pAKT1/2T308 was only partially inhibited. This may suggest that AKT1/2T308 is regulated by another pathway or that the known positive feedback mechanism induced by mammalian target of rapamycin complex 2 (mTORC2) and observed in other hematological malignancies10 is active. Furthermore, while IPI-145 was not toxic to normal control T and B cells, CLL T, B, and natural killer cells were more sensitive to this agent. Further work is required to understand this mechanism and what it will mean to patients.

Although these data confirm BCR signaling was inhibited by IPI-145, there are still a significant number of unanswered questions. Will IPI-145 inhibit CXCR4/CXCL12 signaling and subsequent migration and T-cell (CD40L and interleukin-4 [IL-4]), stromal (BAFF, IL-6), and TLR-mediated signaling as previously shown with idelalisib? Will IPI-145 be superior to idelalisib in patients? Will IPI-145 need to be combined with another agent such as BH3 mimetics or monoclonal antibodies (mAbs) to obtain greater efficacy? In theory, both should synergize well with IPI-145 through the previously mentioned mechanism of retaining CLL cells in the blood, both reducing the anti-apoptotic signaling and providing easy access for mAbs. Finally, is inhibiting 2 isoforms better than inhibiting 1? Might the inhibition of the γ isoform of there is more off-target toxicity to other nonhematological tissues if PI3Kα and PI3Kβ are also inhibited.

In this issue, Dong et al1 investigate in vitro an alternative PI3K inhibitor, IPI-145, which is currently in a phase 3 clinical trial as a monotherapy for CLL (www.clinicaltrials.gov; #NCT02004522). Unlike idelalisib, IPI-145 targets both α and γ isoforms of PI3K that are expressed in leukocytes. In vitro, IPI-145 induced up to ~30% apoptosis of primary CLL cells in a dose- and time-dependent manner, which is similar to published data with idelalisib.1 Importantly, samples that were resistant to ibrutinib were still susceptible to killing from IPI-145 even though BTK exerts its effect both upstream and downstream of PI3K, suggesting PI3K may compensate after BTK/phospholipase C-γ2 mutations/disruption. IPI-145 significantly inhibited signaling with anti-immunoglobulin M at much lower concentrations than idelalisib1,9; however, pAKT1/2T308 and ERK 1/2T202/Y204 were completely abrogated and confirm PI3K inhibition, pAKT1/2T308 was only partially inhibited. This may suggest that AKT1/2T308 is regulated by another pathway or that the known positive feedback mechanism induced by mammalian target of rapamycin complex 2 (mTORC2) and observed in other hematological malignancies10 is active. Furthermore, while IPI-145 was not toxic to normal control T and B cells, CLL T, B, and natural killer cells were more sensitive to this agent. Further work is required to understand this mechanism and what it will mean to patients.

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PI3K, induced by IPI-145 but not idelalisib, in fact lead to more tumor toxicity in vivo? Similarly, might it reduce the effector functions of various immune effectors when used in combination with mAbs? For these answers, we eagerly await the results of the upcoming trials and further in vitro experimentation.

Despite these unresolved questions, this article provides exciting new data and insight into the biology of the PI3K signaling pathway in CLL cells, which will enable the development of more effective drugs for the treatment of this currently incurable disease. Moreover, the more inhibitors we have for the BCR signaling pathway, the more tools we will have to further dissect its critical signaling functions in malignant cells and the more opportunities we will have to explore rational combinations for improved therapeutic efficacy in the future.

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

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4. Wodarz D, Garg N, Komarova NL, et al. Kinetics of therapeutic inhibition of the PI3K pathway in CLL cells, which will enable the more inhibitors we have for the BCR signaling pathway, the more tools we will have to further dissect its critical signaling functions in malignant cells and the more opportunities we will have to explore rational combinations for improved therapeutic efficacy in the future.

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Comment on Velasco-Hernandez et al, page 3597

Targeting HIF function: the debate continues

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In this issue of Blood, Velasco-Hernandez et al1 come to an important and at first sight, unexpected, conclusion that hypoxia inducible factor 1α (HIF-1α) may be a tumor suppressor gene.

Current data suggest leukemic stem cells (LSCs) are adapted to hypoxia, raising the possibility of therapeutic targeting of the transcriptional regulator HIF-1α. Direct oxygen measurement of human marrow shows it has a low overall oxygen partial pressure (~55 mmHg, with an oxygen saturation of 87.5%). Furthermore, perfusion tracer experiments suggest that functional murine hematopoietic stem cells (HSC) are enriched in the lowest perfusion compartment.2 Recent elegant data confirmed what was long suspected: that there is regional variation in vascularization. The central marrow narrow diaphysis is poorly vascularized and enriched for cells staining with pimonidazole, a chemical that makes thiol adducts in a low oxygen environment.3 This, coupled with observations that murine HSCs and human LSCs reside next to the endostium,4,5 support the concept that HSCs and LSCs reside in a particularly hypoxic niche.

The heterodimeric transcriptional regulator HIF, which is composed of α and β subunits, mediates, in large part, adaption to hypoxia. There are 3 different α subunits, HIF-1α, HIF-2α, and HIF-3α, and a common HIF-1β subunit. HIF regulates the expression of many genes, which together allow cells to adapt to hypoxia and a low nutrient environment by switching energy production from oxidative to glycolytic pathways and reducing reactive oxygen species production that could have a deleterious mutagenic impact on the genome and promoting quiescence.2 Although all 3 HIF α subunits are expressed in murine HSCs, genetic manipulation of HIF-1α protein levels demonstrates that HIF-1α promotes murine HSC engraftment and quiescence when tested in transplantation assays and in aged mice.6 In contrast, HIF-2α does not appear to have a similar or additive role in HSCs.7

These observations, coupled with a large amount of literature on HIF function in cancers more generally, have promoted the concept of therapeutic targeting of HIF function. Credence for the notion that HIF-1α may be a therapeutic target in hematologic malignancies has come from in vitro and limited in vivo studies of primary human acute myeloid leukemia (AML) samples, in an experimental murine lymphoma model using the HIF-1α inhibitor echinomycin,8 and in an experimental murine chronic myeloid leukemia model in an HIF-1α−/− background.9 However, there are also data to support a more important role for HIF-2α, rather than HIF-1α, from knockdown studies that show reduced engraftment when 7 human AML samples were tested.10 Knockdown of HIF-2α also reduced short- and long-term engraftment of primary human CD34+ stem/progenitor cells, suggesting more work needs to be done to establish whether there is a therapeutic index for targeting HIF function.

Now add into the mix the paper from Cammenga’s laboratory, which asks the following question: is HIF-1α required for leukemic growth in 3 different murine AML models. They studied 3 AML models: a tetracycline inducible human mixed-lineage leukemia (MLL)–eleven nineteen leukemia (ENL) murine knock-in (KI) model and 2 transplantation models where mice were transplanted with bone marrow cells retrovirally transduced with either...
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