elimination. A CD27-truncated receptor was used that binds CD70, triggering a response in the NK cells but delivering no CD27-based signal to the expressing cell. Expression of the truncated CD27 resulted in a marked decrease in tumor burden in both fibrosarcoma and Eμ-myc–induced lymphoma models in vivo. Importantly, this effect was clearly dependent on NK cells, since elimination of NK cells reverted the growth kinetics of CD27-truncated expressing to that of CD27-negative tumor cells. Similarly, this effect was directly related to CD70, since blockade of CD70 using antibody or genetic deletion ($CD70^{-/-}$) also abrogated the antitumor response. Such loss-of-function experiments are important to demonstrate the link between CD70, NK-cell activation, and tumor elimination. Moreover, the authors provided direct evidence for a CD27-induced NK cells response: expression of the CD27-truncated protein resulted in NK-cell accumulation in tumors and enhanced interferon-γ (IFN-γ) responses by NK cells isolated from the tumor microenvironment. This is an important finding, since NK cells isolated from the blood or noninvolved lymphoid tissues lacked the enhanced responses found at in those at the site of the tumor. Typically, robust NK cell activation requires engagement of multiple activating receptors. The authors experimentally address this point by showing that CD70 cooperated with other activating rectors to enhance IFN-γ production by NK cells in vitro. From a mechanistic standpoint, ligation of CD70 on NK cells resulted in increased Akt signaling, a well-known activation pathway for NK cells. Identification of a key signaling pathway can inform the testing of complementary activating receptor agonists, cytokine receptors, or inhibitory receptor blockade to further optimize NK-cell activation and antitumor responses. To highlight the potential relevance to human disease, the authors employed a $CD27^{+}$ acute lymphoblastic leukemia xenograft model and demonstrated CD70-dependent activation and persistence of adoptively transferred human NK cells. In a hypothesis-generating analysis, the authors also used public gene expression data and report univariate correlations between higher expression of CD27, CD56, and NKG2D (an NK-cell–activating receptor) and improved survival in lymphoma patients. Collectively, this study provides comprehensive data supporting CD70 as an important NK-cell–activating receptor that augments antilymphoma responses.

What does this mean for physicians who treat patients with lymphoma or lymphoma researchers developing new therapies? The findings of this study provide a clear preclinical rationale to test the therapeutic strategy of CD70 coactivation to augment NK-cell antilymphoma responses. While this report is convincing, it will be important to confirm the ability of CD70 to activate NK-cell responses in the presence of a complete immune system and with physiologic expression of its ligand, CD27. In addition, the expression of CD70 and CD27 on other immune cell types (CD4 and CD8 T cells, B cells, and dendritic cells) will need to be considered.[9] Regardless, translation will also require a cautious and thoughtful approach, since CD70 is also expressed (albeit variably) on most types of lymphoma, and ligation has resulted in activation and proliferation of malignant B cells in vitro. Indeed, for these reasons, CD70 has been identified as a therapeutic antibody target being investigated in hematologic malignancies. This concern could be addressed by restricting the testing of CD70 agonists to CD70-negative lymphomas or potentially using trispecific proteins that coordinately engage CD70 and an CD16a (or another NK-cell–selective activating receptor), while the third scFv directs to the lymphoma.

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

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● ● ● LYMPHOID NEOPLASIA

Comment on Erdmann et al, page 310

Might broader be better?

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In this issue of *Blood*, Erdmann et al examine the importance of phosphoinositide 3-kinase (PI3K) in diffuse large B-cell lymphoma (DLBCL) and rationalize dual inhibition of the p110 α and δ isoforms.[1]

DLBCL is the most common form of non-Hodgkin lymphoma; it has an aggressive course that requires immediate treatment. Two main subtypes, activated B cell-like (ABC) and germinal center B cell-like (GCB), were identified on the basis of cell of origin and subsequently further characterized by gene expression profiling; ABC was found to have a worse prognosis. Both the gene signature and next-generation sequencing analyses point to a dependence upon the nuclear factor κB (NF-κB) pathway. Upstream of NF-κB, the B-cell receptor (BCR) signaling pathway has gain-of-function mutations in *CD79A*, *CD79B*, and *MYD88* and increased PI3K pathway signaling.

In cells of hematopoietic origin, the major catalytic isoform expressed is p110 δ (PIK3CD), which is mutated in some patients with lymphoma.[2] On the basis of these

findings, it is unsurprising that PI3K inhibitors have been tested in many lymphomas (reviewed in Blachly et al[3]). In 2014, the US Food and Drug Administration approved a PI3K p110 δ–specific inhibitor, idelalisib, for use in relapsed/refractory chronic lymphocytic leukemia and indolent lymphomas. However, this agent has failed in DLBCL phase 1 studies.

Erdmann et al screened ABC and GCB cell lines with various pharmacologic agents including idelalisib, ibrutinib, the dual p110 α/δ PI3K inhibitor AZD8835, and the AKT inhibitor AZD5363. PTEN-deficient DLBCL cell lines, like most GCB DLBCLs, were sensitive to AKT inhibition. ABC cell lines that were NF-κB–pathway dependent were sensitive to dual p110 α/δ inhibition, using both pharmacologic agents and genetic short hairpin RNA (shRNA) knockdown. AZD8835's activity was confirmed in vivo using cell lines OCI-Ly10 and TMD8 and appropriate patient-derived xenografts (PDXs).

These results suggest that dual inhibition of the p110 α and δ subunits of PI3K in ABC DLBCL models that are BCR and NF-κB dependent should be tested in the clinical setting. The article encourages the molecular characterization of each patient's lymphoma, especially ABC versus GCB, BCR and NF-κB pathway mutations, and PTEN deficiency. This is not the first description of such an idea; Paul et al[4] reported the preclinical success of copanlisib in CD79B- and MYD88-mutated ABC DLBCL models. Several clinical trials are already under way with such agents, including copanlisib and buparlisib.

Of course, these findings should be considered with some caution, given that the results are from panels of cell lines. Although well characterized, these cell lines may have mechanisms of sensitivity and resistance that differ from those of primary tumor cells, and due to a variety of factors, even the PDX models may not be entirely relevant. It is hoped that, as clinical trials are carried out using the prospective molecular characterization proposed here, the role of these models in drug development for DLBCL will be confirmed.

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

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THROMBOSIS AND HEMOSTASIS

Comment on Hudecova et al, page 340

Prenatal diagnosis by droplet digital PCR

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In this issue of *Blood*, Hudecova and colleagues describe a simple, noninvasive assay for prenatal detection of hemophilia by droplet digital polymerase chain reaction (ddPCR) on maternal peripheral blood.[1]

Prenatal diagnosis is a must!
In digital PCR we trust.
With blood from carrier moms,
Alleles are compared, mutant and non.

For a fetus of male sex
With mutant allele excess,
Diagnosis is elemental,
And compared with amnio, so gentle!

Hemophilia in the neonate
Known before the birth date,
Allows for factor injection
For circumcision or C-section.

Whether intron 22 mutation,
Missense, or other alteration,
Carrier blood collection
Is the key to prenatal detection.

Hemophilia is an X-linked disorder caused by deficient or defective factor VIII (hemophilia A) or factor IX (hemophilia B) and is characterized by spontaneous or traumatic bleeding into joints and muscles. Infants born with hemophilia may have circumcision bleeding or life-threatening intracranial hemorrhage, for which factor prophylaxis may be protective.[2] Thus, early diagnosis, preferably prenatal diagnosis, is critical to prevent disabling complications. Currently, hemophilia is diagnosed by the factor VIII or IX activity on a cord blood sample obtained at birth. By this approach, however, a diagnosis may be missed in up to one-third of hemophilia cases, which arise as a spontaneous mutation, or may be unsuspected in up to two-thirds of cases with a family history, if the mother is unaware of her carrier status, even despite a bleeding tendency.[3,4]

The recently established nationwide program, My Life, Our Future (MLOF), to genotype individuals with hemophilia A and B and their female carrier relatives, has begun to alleviate the latter problem.[5] Knowledge of genotypic carrier status is critical to prenatal diagnosis, as prenatal tests for hemophilia are increasingly being developed to test carrier mothers during pregnancy. In 2011, Tsui et al introduced a microfluidic assay for prenatal diagnosis that analyzes maternal plasma DNA for *F8* or *F9* sequence variants, such that an allelic imbalance with more mutant (M) than normal/wild-type (N) allele in maternal carrier plasma indicates an affected child.[6] The latter technique, although highly accurate, is not widely used, as, due to molecular complexity, it cannot detect the most common mutation causing severe hemophilia A, the intron 22 inversion mutation.[7]

In this study, Hudecova and her colleagues employed powerful new genomic techniques, including ddPCR and massively parallel sequencing, to analyze maternal plasma DNA samples from 18 at-risk pregnancies (see figure). Sampling from 18 to 42 weeks' gestation, they correctly diagnosed hemophilia in the fetus of 15 pregnancies, including hemophilia B in 8 and hemophilia A in 7, 3 of which were identified to have the intron 22 inversion mutation. In 3 at-risk pregnancies, no