findings, it is unsurprising that PI3K inhibitors have been tested in many lymphomas (reviewed in Blachly et al^4). 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 AZD3563. 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.

## REFERENCES


## THROMBOSIS AND HEMOSTASIS

**Comment on Hudecova et al, page 340**

### Prenatal diagnosis by droplet digital PCR

**Margaret V. Ragni**

**UNIVERSITY OF PITTSBURGH MEDICAL CENTER; HEMOPHILIA CENTER OF WESTERN PA**

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...

DOI 10.1182/blood-2017-03-770206
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carrier testing and genetic counseling of female members from hemophilia kindreds.

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

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**DOI 10.1182/blood-2017-05-786269**

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**TRANSPLANTATION**

**Comment on Martin et al, page 360**

**Raising the bar for chronic GVHD trials**

**Nicolaus Kröger  UNIVERSITY MEDICAL CENTER**

In this issue of Blood, Martin et al propose a new primary endpoint for clinical studies on chronic graft-versus-host disease (GVHD). Their results from a prospective multicenter observational study are helpful for
Prenatal diagnosis by droplet digital PCR

Margaret V. Ragni