have a higher propensity of disease relapse after treatment with chemo-immunotherapy. Notably, the authors observed an increased resistance to fludarabine, and to a lesser extent B-cell receptor signaling inhibitors, at least for cells carrying selected *RPS15* mutations. Hence, it will be important to study additional potential treatment targets or combinations of therapy for this poor-prognosis group of CLL patients.

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

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Comment on Mottok et al, page 2401

PMBCL: a molecular diagnosis?

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In this issue of *Blood*, Mottok et al[1] demonstrate the utility of a molecular assay that assesses the expression of 58 genes to distinguish primary mediastinal B-cell lymphoma (PMBCL) from diffuse large B-cell lymphoma (DLBCL) by using routinely available formalin-fixed paraffin-embedded tissue (FFPET) biopsies. The results could improve diagnostic accuracy for patients with PMBCL and may have important implications for clinical trial selection and interpretation of clinical outcomes for patients with this rare form of lymphoma.

PMBCL is a unique clinicopathologic subtype of aggressive B-cell lymphoma, the diagnosis of which is dependent on correlating clinical features and pathologic and immunophenotypic features.[2] Despite improvements in the diagnostic criteria that have been developed for many subtypes of non-Hodgkin lymphoma, distinguishing PMBCL from DLBCL and gray zone lymphoma involving the mediastinum is still a challenge when using currently available diagnostic methods.

By leveraging the wealth of gene expression profiling (GEP) data obtained from PMBCL samples,[3,4] the authors sought to establish a molecular assay that would be broadly applicable for routine clinical diagnostic testing. Important criteria were the ability to use nucleic acids obtained from routinely fixed FFPETs and to use a widely available diagnostic platform. The authors addressed this challenge by developing a quantitative gene expression assay that analyzed 58 genes using the Nanostring platform labeled Lymph3Cx.

The Mottok article describes a rigorous study that used a training set composed of 68 cases of PMBCL and DLBCL and an independent validation set composed of 158 cases. The authors used 30 genes to distinguish between PMBCL and DLBCL, 24 of which were overexpressed in PMBCL and 6 of which were overexpressed in DLBCL and represented high discriminative power. The assay also included genes that are represented in Lymph2Cx, which allowed for cell-of-origin (COO) determination in DLBCL not otherwise specified (NOS). Thus, the assay could use molecular signatures to distinguish PMBCL and also provide COO assignments to DLBCL NOS (see figure). The reproducibility of the assay performance in 2 independent laboratories was high, which supports the likelihood of this assay becoming clinically relevant.

Patients with PMBCL demonstrate selective responses to novel therapeutic approaches such as dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, and rituximab (EPOCH-R)[5] or anti-programmed cell death protein 1 antibody[6] vs those therapies for DLBCL. Thus, the ability to more accurately distinguish PMBCL from DLBCL has important implications for patient management.

Potential obstacles in the rapid and large-scale adoption of this assay in routine clinical settings are the availability of the technical platform and the amount of tissue required (core needle biopsies vs excisional lymph node biopsies) for the assay to perform at the levels reported in the Mottok article. Most importantly, the ability to translate the results of the Mottok study is limited by the fact that the assay was developed by using a specific technical platform, and thus the applicability to other GEP methods such as reverse transcriptase multiplex ligation-dependent probe amplification[7] is unknown.

One major diagnostic challenge that is not addressed is the distinction between PMBCL and classical Hodgkin lymphoma (CHL). Given the biologic overlap between these 2 entities, GEP assays such as those described in the Mottok article may provide a more robust and accurate molecular assay to distinguish PMBCL from CHL and provide quantitative gene expression-based cutoff values that could

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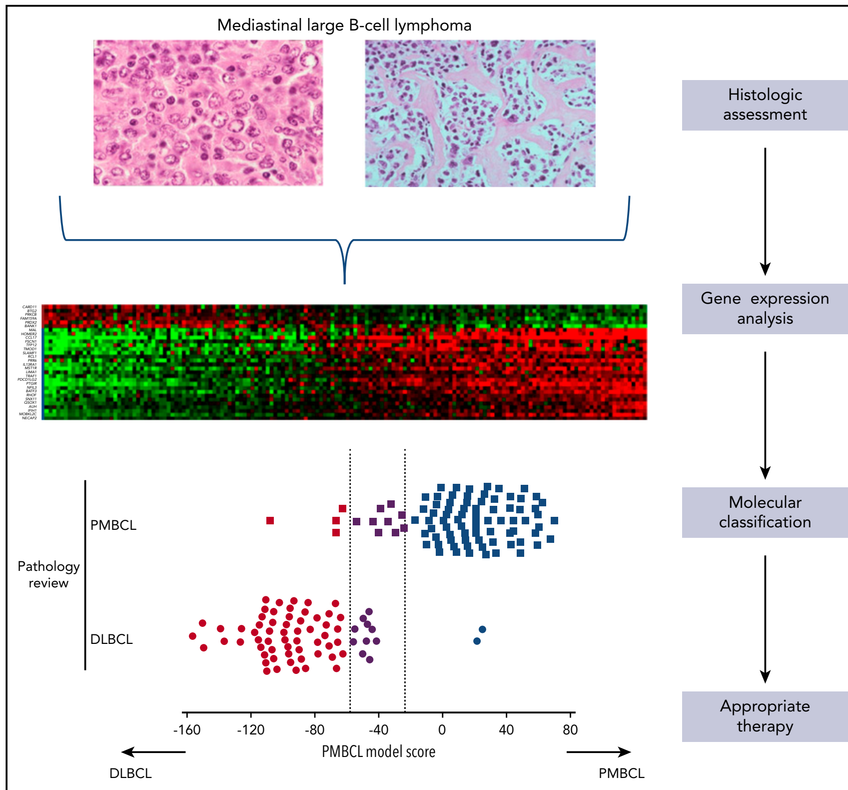

Use of a gene expression-based assay in the molecular classification of large B-cell lymphoma in the mediastinum into PMBCL and DLBCL.

be used to assign diseases that truly represent gray zone lymphomas.

The Mottok study highlights the ongoing development of quantitative robust GEP-based molecular assays such as DLBCL COO classification[8] and assays that may be in current development for classifying peripheral T-cell lymphoma.[9] These add to the expanding armamentarium of molecular assays (eg, circulating tumor DNA, targeted next-generation sequencing panels, epigenetic profiling, exosome analysis) that will be available to the diagnostic team in the near future.

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

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RED CELLS, IRON, AND ERYTHROPOIESIS

Comment on Qu et al, page 2406

Ineffective erythropoiesis of TET2 deficiency

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Studies by Qu et al[1] in this issue of *Blood* provide new mechanistic insight into the ineffective erythropoiesis of TET2 deficiency including selective colony-forming unit–erythroid (CFU-E) hyperproliferation, heightened expression of KIT and *AXL*, and the rescue by KIT and AXL inhibitors of faltered late stage erythropoiesis.

(De)methylation of DNA at specific loci and elements can guide hematopoietic progenitor cell growth and differentiation.[2] If imbalanced, hypermethylation can repress tumor suppressors, whereas hypomethylation can contribute to genomic instability.[2,3] Proteins TET-1, TET-2, and TET-3 function as Fe(II), 2-oxoglutarate- dependent dioxygenases to mediate DNA demethylation. This involves 5mC oxidation to 5hmC, 5fC, and 5caC. Seminal clinical studies have associated deletions and/or mutations within TET2 (4q24) with myelodysplastic syndrome (MDS), myeloproliferative neoplasm, chronic myelomonocytic leukemia, and acute myeloid leukemia.[4] TET2 mutations commonly cluster within catalytic regions (residues

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