This study demonstrates the clinical utility of a targeted gene sequencing panel "the Lymphopanel," which enables the detection of actionable mutations and subtype-enriched gene alterations in diffuse large B-cell lymphoma that will pave the way to precision therapy era for patients with this form of aggressive lymphoma. Clin Cancer Res; 22(12): 2829–31. ©2016 AACC. See related article by Dubois et al., p. 2919

In this issue of Clinical Cancer Research, Dubois and colleagues (1) demonstrate the clinical utility of a targeted gene sequencing panel for diffuse large B-cell lymphoma (DLBCL). DLBCL is an aggressive cancer and represents the most common form of lymphoma in adults, accounting for 30% to 40% of newly diagnosed non-Hodgkin lymphoma (2). Importantly, although immunochemotherapies have improved the outcome of many patients with DLBCL, a subset of patients with relapsed or refractory disease suffer from poor outcomes. Extensive gene expression profiling (GEP) studies over the last decade have led to the recognition of three main molecular subtypes: germinal center B-cell like (GCB), activated B-cell like (ABC; ref. 3), and primary mediastinal B-cell lymphomas (PMBL; ref. 4). Subsequent studies revealed that ABC subtypes are characterized by aberrant activation of the NF-κB pathway, while PMBL demonstrate JAK–STAT activation. Indeed, current NCCN Clinical Practice Guidelines in Oncology (5) advocate the evaluation of DLBCL for subclassification into GCB type from non-GCB and PMBL using either immunohistochemical methods or GEP. Despite these advances in our understanding of the pathobiologic basis of DLBCL, the immediate impact on clinical management and translation to the bedside has been lacking. Recent advances in sequencing technology have provided novel insights into the genetic alterations that are present in these subtypes and the identification of recurrent somatic mutations that are enriched in particular subtypes (6, 7). Dubois and colleagues (1) demonstrate the clinical utility of a targeted gene sequencing panel "the Lymphopanel," which enables the detection of actionable mutations and subtype-enriched gene alterations.

Despite a significant genetic heterogeneity within subtypes of DLBCL, the premise of translating the genetic signatures by leveraging next-generation sequencing technology for clinical application is a welcome development. Using a custom panel designed to identify mutations in 34 genes that have been previously implicated in DLBCL, the authors evaluated the tumor DNA of a large cohort of patients (n = 215) enrolled in a prospective, multicenter randomized clinical trial (LNH-03B LYSA). The results thus allow the authors to gain unique information for assessing the prognostic value of the genetic alterations. Further adding to the power was the availability of GEP-based cell of origin molecular classification. This provided the opportunity to correlate the prognostic impact on molecularly defined subtypes of DLBCL.

The 34 genes covered 8 pathways important in lymphomagenesis including immunity (CIITA, B2M, TNFRSF14, and CD58), NOTCH (NOTCH1 and NOTCH2), apoptosis/cell cycle (MIFHAS1, XPO1, MYC, CDKN2A/B, FOXO1, TP53, GNA13, and BCL2), NF-κB (TNFAIP3, MYD88, PIM1, CARD11, IRF4, and PRDM1), epigenetic regulation (EZH2, KMT2D, EP300, MEF2B, and CREBBP), MAFK (BRAF), JAK-STAT (SOCS1 and STAT6), and BCR (CD79A/B, ITKPKB, TCF3, and ID3).

Importantly, gene mutation frequencies were variable among the patients, from 1 to 124. Furthermore, the results confirmed the enrichment of certain mutations in the 3 subtypes. For example, mutations in genes of the NF-κB pathways (45% of total variants) and epigenetic regulation (20.2% of total variants) were dominant in ABC subtypes. In GCB subtypes, mutations in genes in the epigenetic pathway (32.3% of total variants) and the apoptosis/cell cycle pathway (26.3% of total variants) were common. PMBLs were characterized by mutations in the JAK/STAT pathway (29.1% of total variants), immunity (20.9% of total variants), and apoptosis/cell-cycle pathways (20.9% of total variants). Of interest, PMBLs were characterized by a higher number of variants per sample than the other subtypes. Despite the use of a limited number of genes in the "Lymphopanel," gene alterations not previously reported in PMBL, such as ITKPKB, MIFHAS1, and XPO1, were seen to be highly enriched in PMBL.

Given the current and increasing interest in personalizing selection of therapies to those that target cancer-specific...
aberrations, it is important that the "Lymphopanel" included genes for which disease-associated mutations could provide information relevant to therapy. Examples of actionable mutations include those impacting MYD88 and CD79B, which is associated with significant response to ibrutinib, a BTK inhibitor (8). In contrast, CARD11 and TNFAIP3 mutations are associated with decreased response to both ibrutinib and sotrastaurin, a protein kinase C inhibitor (9). Although IRF4 lacks a recognized therapeutic inhibitor, ABC subtype DLBCLs have been shown to be addicted to IRF4 and confer response to the immunomodulator, lenalidomide (10).

IRF4 mutations were observed in 7.9% of DLBCL representing 13.6% of ABC subtype, 11.1% of PMBL, and 4.8% of GCB subtype. In addition, high frequency mutations of PIM1 were present in 33.3% of ABC type DLBCL, suggesting the opportunity to evaluate PIM1 inhibitors (11) as a therapeutic option.

Targetable gene mutations in the GCB type cohort included BCL2, which was present in 24.1% of cases. Although BCL2 can be targeted by BH3-mimetics, the mutations identified in this cohort did not impact the BH3 domain, suggesting that the BH3 mimetic activity would not be hampered in these patients. Genes associated with epigenetic function (EZH2, CREBBP, KMT2D, and MYC) were highly represented in GCB types. More than 18% harbored EZH2 mutations highlighting the potential relevance in utilizing EZH2 inhibitors (12) for a subset of GCB type of DLBCL. Figure 1 summarizes the genetic signatures characteristic of DLBCL subtypes. Rare cases with mutations that are highly actionable were identified such as V600E BRAF and NOTCH1.

These studies demonstrate the feasibility of leveraging mutational signatures of subtypes of DLBCL to obtain information for the selection of targeted therapies and correlation with prognosis and pave the way for precision medicine for patients with this aggressive form of lymphoma.

Disclosure of Potential Conflicts of Interest
K.S.J. Elenitoba-Johnson is an uncompensated consultant/advisory board member for Genomenon. No potential conflicts of interest were disclosed by the other author.

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