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Diffuse Large B-cell Lymphoma Classification Tied Up Nicely with a “String”

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Summary

Molecular classification of diffuse large B-cell lymphoma (DLBCL) is a critical biologic, prognostic, and predictive distinction. Since the discovery of two molecular types of DLBCL in otherwise morphologically indistinguishable cases, numerous methodologies for making this distinction have been published. A method and technical platform may now be reaching consensus.

In this issue of *Clinical Cancer Research*, Veldman-Jones and colleagues describe their technical experience using the Nanostring nCounter^R system to develop a gene expression assay to make the distinction between 2 molecular types of diffuse large B-cell lymphoma (DLBCL): activated B cell (ABC-DLBCL); germinal center B cell (GCB-DLBCL); and a third unclassified group with intermediate characteristics (UNC-DLBCL) (1). The authors constructed a model of 145 genes differentially expressed in the ABC and GCB types culled from a variety of DLBCL gene expression profiles (GEP) within the literature. The model was validated on a training set of nine cell lines and tested in a compilation of commercially available tissue samples including, formalin-fixed paraffin embedded (FFPE; n=14), fresh frozen (n=36), and flash-frozen (n=24) biopsies. This model was applied using the Nanostring nCounter system (Seattle, WA) to identify the DLBCL subgroups. Additionally, they compared DLBCL type between time of diagnosis and matched relapsed biopsies in 18 patients suggesting that type remains consistent in most cases. Arriving at an accurate consensus methodology in the cell of origin distinction is crucial for moving forward DLBCL patient management and its significance cannot be overestimated.

First described by Alizadeh and colleagues in 2000, the cell-of-origin (COO) classification of DLBCL took a morphologically indistinguishable set of diseases and identified 2 different types based on GEP in 60 cases using a customized competitive microarray chip known as the “Lymphochip” and snap-frozen, pre-treatment tissue biopsies (2). In that seminal work, hierarchical clustering was used to define gene “signatures” that were associated with overall and progression-free survival (OS and PFS)—with the ABC-DLBCL having a poorer outcome and a GEP signature similar to a post-germinal center (activated) B

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Disclosure of Potential Conflicts of Interest

L.M. Rimsza is part of a research consortium that may be listed as a co-inventor on a provisional patent regarding Nanostring technology, which is owned by the NCI of the NIH.

cell. In contrast, the GCB-DLBCL has a better outcome and a GEP signature similar to germinal center B cells. Subsequently, Rosenwald and colleagues in 2002, using the Lymphochip and snap-frozen biopsies, analyzed a larger cohort of 240 cases to confirm the presence of ABC and GCB signatures as well as definition of an unclassified group (at that time termed “Type 3”) (3). Excitement was palpable as the field began to clarify the long-observed clinical heterogeneity of the disease. Immediately, it became urgent to accurately type cases in order to find sense in any further lymphoma research. In 2003, Wright and colleagues published an algorithm using snap frozen tissues and Affymetrix microarrays, which provided some order by identifying a key gene set and by use of a commercially available microarray chip to molecularly classify DLBCL into COO subgroups (4). This general method was slightly modified and used again by the same research group in Lenz and colleagues in 2008 to classify 233 additional DLBCL cases treated with immunochemotherapy, which demonstrated the continued prognostic significance of the molecular type distinction in the modern treatment era (5).

In 2004, the first of several IHC assays was described in response to the desire for an inexpensive, relatively low-tech method useful for studies using FFPE tissues (FFPET) such as those processed in routine clinical practice (6). Subsequently, several other IHC studies based on additional antibodies and cut-points were reported with agreement between IHC and GEP of 83-93% (7, 8). A draw-back to the IHC studies, however, is the dichotomous distinction between GCB and non-GCB, with the latter category presumably including both ABC-DLBCL and UNCDLBCL. Over the next few years, several publications employed different methods for GEP, reverse transcriptase polymerase chain reaction, cDNA-mediated Annealing, Selection, extension, and Ligation, or quantitative nuclease protection assays applied to both frozen or FFPE tissues and cohorts of patients treated with chemotherapy or immunochemotherapy (9-11). These studies demonstrated different analytic performance and conflicting prognostic correlations, calling into question the clinical significance of the distinction. Confusion plagued the field as the number of methods increased and the prognostic significance came into question.

In the meantime, biological studies continued to emphasize the substantial genomic, genetic, epi-genetic, microRNA and other differences between the types such that the relationship between the “types” is more like two separate diseases rather than subtypes of the same disease. Identification of different mutational profiles in addition to the over-expressed signaling pathways spawned efforts for differential therapy. In at least 2 completed and published studies, the molecular type became not only a prognostic, but also a predictive distinction, with many more studies underway with drugs such as ibrutinib, bortezomib, and lenalidomide.

Given the diagnostic, prognostic, and predictive significance of the COO distinction, an accurate, reliable, and widely-accepted assay for the field is of utmost importance. Such an assay should be applicable to FFPET, sensitive, specific, and reproducible between laboratories and operators. The assay should be able to both accommodate phenotypic variations within types and result in two categories as well as identifying patients where the confidence is low (UNC-DLBCL). Multi-analyte, multiplexed molecular assays are more likely than IHC to have the breadth of reagents needed to make these distinctions accurately.

Furthermore, the assay must be forgiving of the partially degraded mRNA characteristically found in FFPET, not technically burdensome, and standardizable. Table 1 summarizes a few of the differences between the various platforms previously used to classify DLBCL. While IHC may be the least expensive and most suitable for low tumor content samples, the cost is offset by the lower accuracy and binary results. The nCounter system appears to fulfill all needed criteria due to its demonstrated utility in FFPET, documented inter-laboratory reproducibility, and FDA-clearance of the platform for the ProSigna breast cancer assay.

In February 2014, Scott and colleagues described the “Lymph2Cx” assay for classification of DLBCL using the Nanostring nCounter system (12). The study demonstrated outstanding results at multiple sites with a parsimonious 20-gene signature trained and validated on “gold standard” cases from the original Rosenwald and Lenz case series that had undergone expert Pathology consensus review diagnostic. This assay was subsequently selected as the companion diagnostic to be evaluated by the FDA in context of the phase III ROBUST clinical trial sponsored by Celgene (CC-5013-DCL-002). Veldman-Jones and colleagues propose another 145-gene model to demonstrate DLBCL classification on the Nanostring system. The Lymph2cx method used a weighted sum of 15 genes to directly emulate the predictor score defined in Lenz and colleagues and trained on 181 heavily analyzed samples, with cut-points between types taken directly from that predictor. The proposed predictor uses an unweighted average of 145 genes found to be discriminatory between the ABC and GCB types to produce a predictor score, with cut-points chosen empirically based on the results of their predictor on a set of samples previously classified according to type. However, the expense of a large probe set, training the algorithm on immortalized cell lines, small patient sample size, and lack of an external validation cohort may not yield any advantage over the already published Lymph2Cx assay. No direct comparison with the precedent paper by Scott and colleagues is presented. However, both Nanostring-based studies indicate that this non-enzymatic, solution phase hybridization, fluorescent barcode-labeled method, can become the tool needed to move our biologic knowledge of DLBCL into a robust assay for appropriately selecting ABC or GCB patients for well designed clinical trials. After nearly a decade and a half, to paraphrase an old saying, the molecular classification of DLBCL can now be tied up with a “string.”

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Table 1

Methods comparison for determining DLBCL molecular subtype.

Method	Manufacturer	Number of genes	Accuracy vs. gold standard	Useful in FFPET	Inter-lab reproducibility	Expense
Digital array	Nanostring	10-100s	+++	+++	+++	++
Oligonucleotide array	Affymetrix	1000s	+++	++	Not tested	+++
DASL	Illumina	1000s	Not tested	+++	Not tested	+++
Multiplex RT-PCR	PrimerDx	1-20	++	+++	Not tested	++
qNPA	HTG-Molecular	1-48	++	+++	Not tested	++
IHC	Various	1-10	++	+++	+	++

DLBCL = diffuse large B cell lymphoma

FFPET = formalin-fixed, paraffin-embedded tissue

Affy - Affymetrix

DASL = cDNA-mediated Annealing, Selection, extension, and Ligation

RT-PCR = reverse transcriptase-polymerase chain reaction

qNPA = quantitative nuclease protection assay

IHC = immunohistochemistry

+++ = high; ++ = moderate; + = low