

# DLBCL Coast to Coast

By Melania Tesio

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*Integrating genomic, transcriptomic, functional, and clinical data from 1001 patient samples, a new study provides the first comprehensive characterization of diffuse large B-cell lymphoma.*

**D**iffuse large B-cell lymphoma (DLBCL) is the most common lymphoid malignancy in adults. This tumor, highly heterogeneous from a genetic and clinical point of view, is believed to originate from either activated B cells (ABC subtype) or mature B cells transiting germinal centers within secondary lymphoid organs (GCB subtype).<sup>1</sup> Although long-term remission following standard chemotherapy is achieved in nearly half of DLBCL cases, most patients who relapse do not survive. In clinical practice, risk stratification and treatment are based on clinical models for prognostication, such as the International Prognostic Index, which however provide limited insight into the genetic basis of the disease, its pathogenesis, or potential therapeutic targets. Transcriptomic analyses, instead, are more apt to identify molecular subgroups/pathways for therapeutic intervention. Yet, not all transcriptomic studies provided molecular signatures robustly correlating with clinical outcomes.<sup>2</sup> Complicating this scenario, survival-predicting genes lack overlap across different studies. In addition, whereas next-generation sequencing studies highlighted the genetic heterogeneity of DLBCL, they could not identify the full mutational spectrum, mostly owing to limited patient samples that hindered the discovery of rare but important transforming lesions.

By integrating genomic, transcriptomic, functional, and clinical data from 1001 DLBCL samples, Reddy et al<sup>3</sup> provided the first comprehensive characterization of this disease (Fig. 1). In a Herculean effort, recently published in *Cell*, the authors first performed whole-exome sequencing of 1001 clinically annotated DLBCL samples and 502 paired germline DNA samples. This exhaustive screening allowed the authors to identify 150 recurrently mutated genes. Remarkably, although the cohort size allowed 90% power for identifying mutations in 5% of the patients, only 27 out of the 150 genes identified had not been previously implicated in the disease. They included *KLHL14*, *MGA*, and *SPEN*, the most frequently altered gene among the newly identified ones. Also known as *SHARP*, *SPEN* encodes a transcriptional repressor previously shown to regulate marginal zone B cell development via inhibition of the NOTCH/RBPJ pathway. As such, *SPEN* mutations have been recently observed in some cases of marginal zone B cell lymphoma,<sup>4</sup> where they were predicted to truncate the RBPJ-binding domain of the protein and thus to affect NOTCH2 signaling. Whereas truncating mutations were also observed by Reddy et al, their functional consequences in DLBCL might not strictly relate to aberrant NOTCH2 signaling, given that knocking out NOTCH2 did not impact cellular growth. In DLBCL cells, instead, *SPEN* lesions might alter the cellular epigenetic state, for instance by affecting the domains that interact with the histone deacetylase HDAC1 or the nuclear receptor corepressor SMRT,<sup>5</sup> possibilities worthy of further investigation.

To functionally validate the mutations identified, Reddy and colleagues next complemented their exome sequencing data with a genome-wide CRISPR screen targeting 19,050 protein-coding genes. This analysis revealed that 1956 genes are essential in DLBCL as their silencing impacted cellular growth. They were enriched in the genetic drivers identified by exome sequencing, such as *MGA* and *NCOR1*, which were validated as DLBCL suppressors, since their CRISPR-mediated knockout enhanced cellular viability. Conversely, driver genes such as *PAX5*, *HIST1H1E*, and *SF3B1* whose knockout reduced cellular viability were validated as essential oncogenes. Importantly, among them the authors identified 9 genes already known to be direct therapeutic targets of existing drugs, for example *XPO1*, *BTB*, and *SF3B1*.

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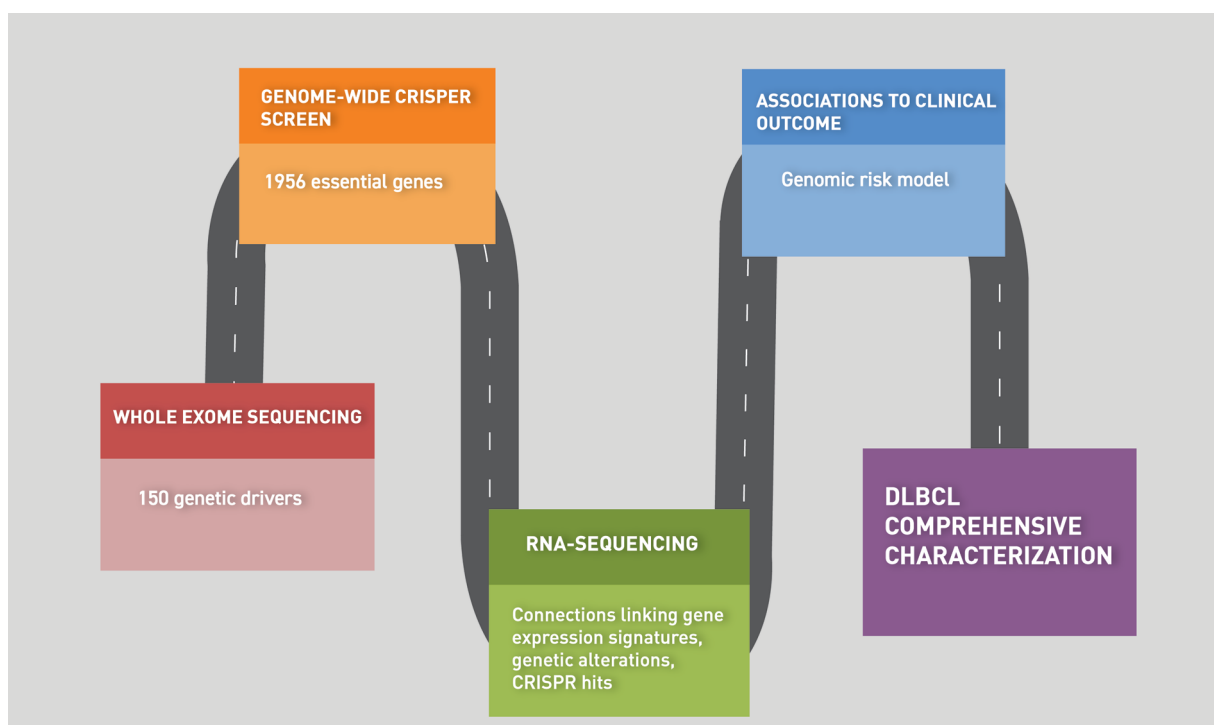


Figure 1. Reddy et al roadmap providing a comprehensive DLBCL characterization.

To further connect genetic alterations and gene expression signatures, the authors performed RNA sequencing on 775 DLBCL samples. Thanks to a bioinformatic analysis integrating mutations, copy-number alterations, CRISPR scores, and gene expression profiles, the researchers could link distinct mutations to specific gene signatures. Confirming expected connections (such as *MYC* and RNA metabolic processes), the researchers revealed novel interesting associations (such as a link between *NOTCH2* and cholesterol biosynthesis) as well as a lack of association between certain previously identified gene signatures (ie, stromal and immune gene signatures) to the overall mutation burden. To link these scenarios to clinical outcome, the authors next performed a multivariate analysis integrating patient survival data with multiple combinations of genetic drivers and gene expression markers. This strategy enabled Reddy et al to construct a genomic risk model, whereby 39 distinct combinations of genetic alterations and gene expression markers predict a specific death risk score. For example, alterations of the *MYC* gene combined with *MYC* expression defined patients with the worst prognosis, whereas a GCB signature combined with *CD70* alterations defined patients with the most favorable outcome. Of note, when validated on an independent test set, this genomic risk predictor outperformed other prognostic models, based either exclusively on genetic alterations, exclusively on gene expression

profiles or exclusively on clinical features, such as the International Prognostic Index.

While it might be challenging to integrate gene expression profiling and mutational analysis of more than 30 genes in everyday clinical practice, the study by Reddy et al offers an unprecedented coast-to-coast journey across the biology of one of the most common lymphoid malignancies, providing comprehensive data to model disease development and understand the biology of poor-prognosis DLBCL.

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