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Introduction: Sequencing studies identified mutational drivers in diffuse large B cell lymphoma (DLBCL), capturing outcome difference in previously unrecognized patient subsets. However, the lack of routine-applicable genomic approaches limits translation of such information to the clinic. Currently, molecular prognostication consists in cell of origin (COO) determination by the Lymph2Cx NanoString assay. We recently developed two independent prognostic signatures incorporating genes reflecting the COO, the activation of pivotal oncogenic pathways, and the composition of tumor microenvironment (TME). We aimed this study at examining the prognostic strength of a model combining the performance of each signature and developing a comprehensive NanoString assay rapidly transferable to the clinic for prognostic purposes.

Methods: The expression of the genes was measured by the NanoString nCounter Analysis System using customized probes for 73 genes, including 15 COO genes, 6 additional oncogenic genes (*MYC*, *BCL-2*, *NFKBIA*, *PIK3CA*, *PTEN*, *STAT3*), 47 TME genes, and 5 housekeeping genes. The analysis was performed on 175 newly diagnosed, nodal DLBCL, homogeneously selected from the RHDS0305 and DLCL04 trials. Patients had comparable clinical features and double-hit cases were excluded. Heatmaps, Kaplan–Meier survival estimator, tree-based survival model, and P values were produced by 'R' statistical software. Long-rank test was used to compare overall survival (OS) and progression-free survival (PFS) among groups. Multivariate analysis was constructed through the Cox proportional hazards regression model.

Results: Based on the expression of the COO and oncogenic genes, a tree-based survival model stratified patients into subgroups showing significantly different survival, with *MYC*, *BCL-2* and *NFKBIA* holding additional prognostic power based on their high (H) or low (L) expression. The TME panel identified a lower gene expression cluster (C3) with significantly worse survival than those at intermediate (C2) and higher expression (C1). Integration of COO-, TME-, and *MYC/BCL-2/NFKBIA*-based data produced a new survival risk categorization of DLBCL. The high-risk category, showing the worst outcomes, includes ABC/H/C1-2-3, ABC/L/C3 and GCB/H/C3 cases; the intermediate-risk category comprises ABC/L/C2, GCB/H or L/C1 and UN/H or L/C1 or C3 cases; whereas the low-risk category contains GCB/H or L/C2, GCB/L/C3, UN/H/C1 or C2 and UN/L/C2 or C3 cases, with longer survival. An unsupervised clustering analysis was also performed based on the expression of the entire 74-gene panel and stratified cases into four clusters with significantly different OS (p=0.011) and PFS (p=0.009). In particular, cluster 1 and 4 showed significantly worse survival than cluster 2 and 3 (Figure 1), and a multivariate Cox analysis indicated that the prognostic performance of the panel overcomes the IPI score. Finally, such model was also validated "in silico" using a gene expression profiling dataset (GSE10846 and GSE98588) relative to a cohort of 146 DLBCL patients uniform[illegible] selected according to R-CHOP treatment.

Conclusions: This study supports the idea that DLBCL heterogeneity involves both tumor and TME, resulting in diverse transcriptional subtypes with distinct outcomes and, putatively, diverse biology. Our integrative analysis prompts the development of a new survival categorization outperforming current prognostic risk-assessment. Moreover, the applicability of a unique Nanostring-based assay to routine biopsies may facilitate the stratification of patients at diagnosis and their inclusion in future trials exploring novel therapeutic approaches.

Keywords: diffuse large B-cell lymphoma (DLBCL); gene expression profile (GEP); prognostic indices.

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CONCORDANCE BETWEEN IMMUNOHISTOCHEMISTRY AND GENE EXPRESSION PROFILING SUBTYPING FOR DIFFUSE LARGE B-CELL LYMPHOMA IN THE PHASE 3 PHOENIX TRIAL

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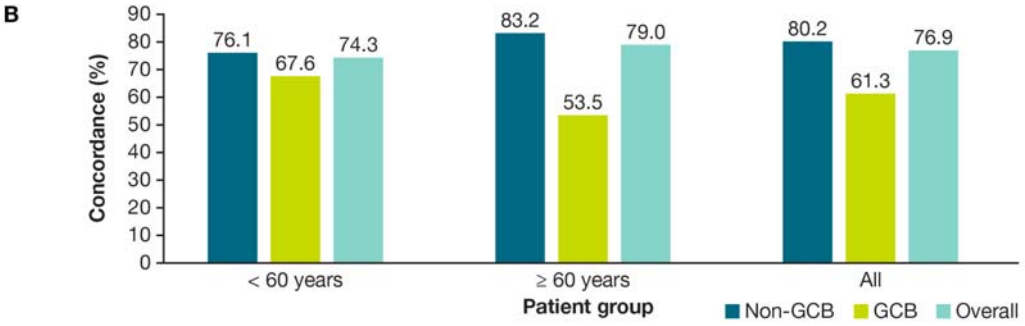
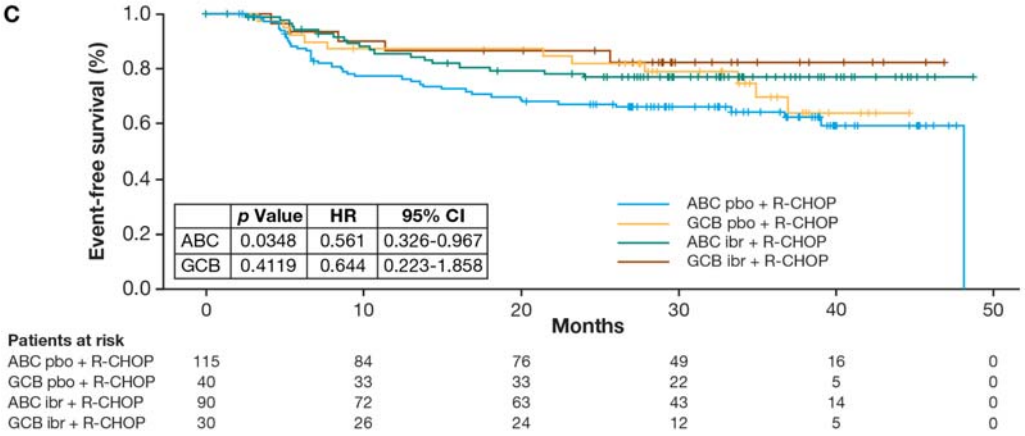
Introduction: Diffuse large B-cell lymphoma (DLBCL) can be classified based on cell-of-origin (COO) into germinal center B-cell–like (GCB), activated B-cell–like (ABC), and unclassified (UNC) subtypes by gene expression profiling (GEP), and GCB and non-GCB subtypes by immunohistochemistry (IHC). In the phase 3 PHOENIX trial (NCT01855750) that enrolled untreated patients (pts) with non-GCB DLBCL by IHC, ibrutinib (ibr) + R-CHOP did not improve event-free survival (EFS) vs placebo (pbo) + R-CHOP in the intent-to-treat (ITT,

Figure. (A) Number of Calls by IHC or GEP; (B) Overall, Non-GCB, and GCB Concordance (%); (C) EFS in ABC and GCB DLBCL in Patients < 60 Years

A

		All Patients		Age < 60 Years		Age ≥ 60 Years	
		EdgeSeq COO GEP					
		GCB	Non-GCB	GCB	Non-GCB	GCB	Non-GCB
Hans-based IHC	GCB	119	75	68	32	51	43
	Non-GCB	182	735	91	279	91	456





Patients at risk

ABC pbo + R-CHOP	115	84	76	49	16	0
GCB pbo + R-CHOP	40	33	33	22	5	0
ABC ibr + R-CHOP	90	72	63	43	14	0
GCB ibr + R-CHOP	30	26	24	12	5	0

non-GCB by IHC) or ABC (by GEP) populations; however, an increase in EFS and overall survival with ibr was seen in pts < 60 years (yrs), but not in pts ≥ 60 yrs due to increased toxicity in elderly pts. This work aimed to determine the concordance between IHC and GEP for DLBCL subtyping and outcomes related to subtypes.

Methods: Baseline paraffin-embedded, formalin-fixed tissue samples were used to confirm non-GCB DLBCL by Hans-based IHC (Dako pharmDx™ kit) at a central laboratory. Available tumor samples were retrospectively analyzed for ABC subtype by GEP (HTG EdgeSeq DLBCL COO Assay). The concordance was evaluated by comparing non-GCB calls by IHC with ABC + UNC by GEP or GCB calls between IHC and GEP. Survival outcomes were compared between GEP subtypes in each arm and across study arms.

Results: In all screened pts, 1111/1336 (83.2%) samples also provided evaluable GEP results; the concordance between GEP and IHC was 80.2% for non-GCB and 61.3% for GCB calls, resulting in an overall concordance of 76.9% (Figure), with 73.7% of non-GCB samples (by IHC) being identified as ABC by GEP. In pts < 60 yrs (n = 506), the concordance for non-GCB, GCB, and overall was 76.1%, 67.6%, and 74.3% respectively. In 747 evaluable samples from 838 enrolled non-GCB pts, 75.9% were ABC by GEP; 17.2% were GCB and 6.8% UNC. In both ITT and age < 60 yrs populations, EFS rate in GCB DLBCL by GEP was higher vs ABC in either arm, although the difference was not statistically significant and even smaller in the ibr arm. When comparing the two arms in the ITT population, EFS was similar between arms regardless of COO. In pts < 60 yrs, EFS was better with the addition of ibr to R-CHOP in ABC pts (HR 0.56 [95% CI, 0.33-0.98]; p = 0.0348; Figure); the difference between arms was not statistically significant in GCB (HR 0.64 [95% CI, 0.22-1.86; p = 0.4119) or UNC (HR 1.12 [95% CI, 0.22-5.97]) subtypes as numbers were small.

Conclusions: The overall concordance between non-GCB by Hans-based IHC and GEP using the EdgeSeq DLBCL COO Assay was 74-77% in the ITT population and age-related subgroups, even with centralized testing. Although only 75.9% of enrolled pts were the ABC subtype, the addition of ibr improved outcomes in pts < 60 yrs in both IHC-based non-GCB or GEP-based ABC DLBCL.

Keywords: gene expression profile (GEP); ibrutinib; immunohistochemistry (IHC).

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LONGITUDINAL ANALYSES OF DIAGNOSTIC-RELAPSE BIOPSIES OF DIFFUSE LARGE B CELL LYMPHOMA SUGGEST THAT RELAPSE IS MEDIATED BY DISTINCT MECHANISMS IN ABC AND GCB LYMPHOMA

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Introduction: Although diffuse large B cell lymphoma (DLBCL) can be cured using immuno-chemotherapy, 40% of patients experience relapse or refractory disease. Large-scale profiling studies have mainly focused on DLBCL at diagnosis with a limited number of longitudinal studies and no compelling biomarkers linked to relapse identified. To address this, we utilized a multifaceted approach integrating transcriptomic and intratumoral T-cell repertoire analyses in paired diagnostic/relapse tumors to enable identification of signaling pathways and microenvironmental changes underlying disease relapse.

Methods: We retrospectively collected archival paired diagnostic/relapse tumor biopsies from 38 *de novo* DLBCL patients (stage I-IV, 38-89 years old) treated with rituximab-based immuno-chemotherapy. We performed gene expression profiling (GEP) and T-cell repertoire analysis using the Ion AmpliSeq Transcriptome Kit and TCR-β sequencing (immunoSEQ), respectively. Cell-of-origin (COO) classification was performed by the Lymph2Cx assay on NanoString to distinguish activated B-cell-like (ABC) and germinal center B-cell-like (GCB) subtypes.

Results: COO remained stable from diagnosis to relapse in >90% of pairs. In examples where we observed a switch in COO between diagnosis/relapse, targeted-seq analysis revealed some shared mutations suggesting that relapse tumors originated from a common ancestral clone. Our global GEP of 17 ABC-ABC and 11 GCB-GCB pairs identified 163 and 136 genes that were differentially expressed in ABC and GCB relapse tumors relative to their matched diagnostic biopsies respectively, with minimal overlap. Gene set enrichment analysis showed that ABC and GCB relapses are potentially mediated via different mechanisms, with tumor growth and proliferation signatures enriched in ABC relapse, compared with adaptive immunity-related signatures accompanying GCB progression. In parallel, we assessed the dynamics of the T-cell repertoire in paired biopsies observing a