Test-the-test: Clinical utility of comprehensive whole exome and transcriptome sequencing (WES/RNA-seq) for lymphoma patients

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Abstract 1754

Introduction

Due to the rapidly evolving landscape of targeted therapies, there is an unmet need for comprehensive molecular profiling to guide treatment decisions for patients with lymphoma. To meet this need, we designed a pilot study (NCT05464823) to assess the feasibility and turnaround time (TAT) of a comprehensive WES and whole transcriptome sequencing (RNA-seq) assay (BostonGene Tumor Portrait™ test) in large B-cell lymphoma (LBCL) patients for clinical decision-making.

References
1. Boltežar L, Prevodnik VK, Perme MP, Gašljević G, Novaković BJ. Comparison of the algorithms classifying the ABC and GCB subtypes in diffuse large B-cell lymphoma. Oncol Lett. 2018 May;15(5):6903-6912.
2. Kotlov N, et al.: Clinical and Biological Subtypes of B-cell Lymphoma Revealed by Microenvironmental Signatures. Cancer Discov. 2021 Jun;11(6):1468-1489.
3. Wright GW, et al.: A Probabilistic Classification Tool for Genetic Subtypes of Diffuse Large B Cell Lymphoma with Therapeutic Implications. Cancer Cell. 2020 Apr 13;37(4):551-568.e14.

Methods

Patients aged ≥18 years with histologically documented LBCL requiring therapy were eligible. Formalin-fixed, paraffin-embedded tissues underwent WES and RNA-seq with concomitant germline DNA sequencing using peripheral blood or saliva. Samples with <20% tumor purity and median coverage of <142x for tumor and/or <95x for normal samples were labeled as quantity not sufficient (QNS). To date, 49 patients received WES and RNA-seq **(Fig. 1)**. For 45 cases, genomic and transcriptomic profiles were analyzed to uncover clinically relevant/actionable events and the findings were summarized in Tumor Portrait™ test reports. These reports were supplemented with selected treatment options, matched clinical trials, and biomarker annotations. Among these final reports, three contained limited results due to QNS samples.

Results

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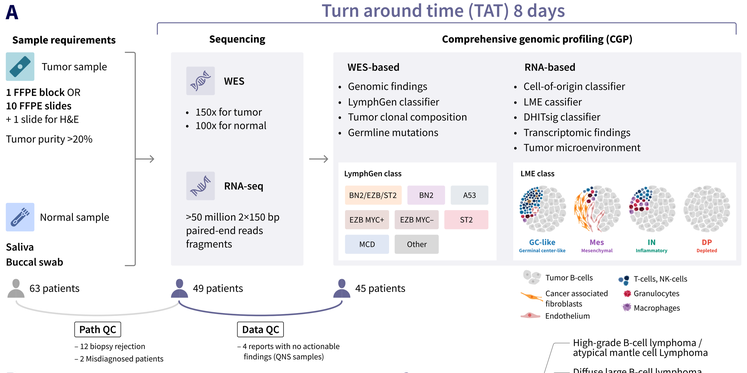
Figure 1. Pipeline and cohort overview



B

Cohort overview

Age, median		**Sex**	
Age	64 (34–86)	Male	22/45 (48.89%)
		Female	23/45 (51.11%)
Disease stage			
II	3/45 (6.67%)	IV	24/45 (53.33%)
IIE	2/45 (4.44%)	N/A	2/45 (4.44%)
III	6/45 (13.33%)		
III/IV	1/45 (2.22%)	N/P	7/45 (15.56%)
Specimen site		**Disease status**	
Lymph node	18/45 (40.00%)	ND	28/45 (62.22%)
Extranodal	27/45 (60.00%)	RR	17/45 (37.78%)
Clinical history			
Prior lines of therapy for RR pts, median (range)		2 (1–7)	
Prior CAR-T treatment in all patients		10/45 (22.22%)	

C

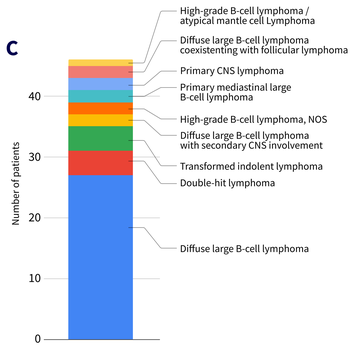


Figure 1. Pipeline and cohort overview. **A.** Pipeline for generating custom reports. For the trial, custom versions of the BostonGene Tumor Portrait™ tests containing comprehensive overview of actionable genomic and transcriptomic findings were delivered with a median TAT of 8 days. **B.** Clinical characteristics of the patients enrolled in the trial. Staging results were not provided (N/P) or not applicable (N/A) for 12 patients in total. **C.** Histological subtypes included in the cohort. ND - newly diagnosed; RR - relapsed/refractory.

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Figure 2. Overview of molecular findings and actionable events

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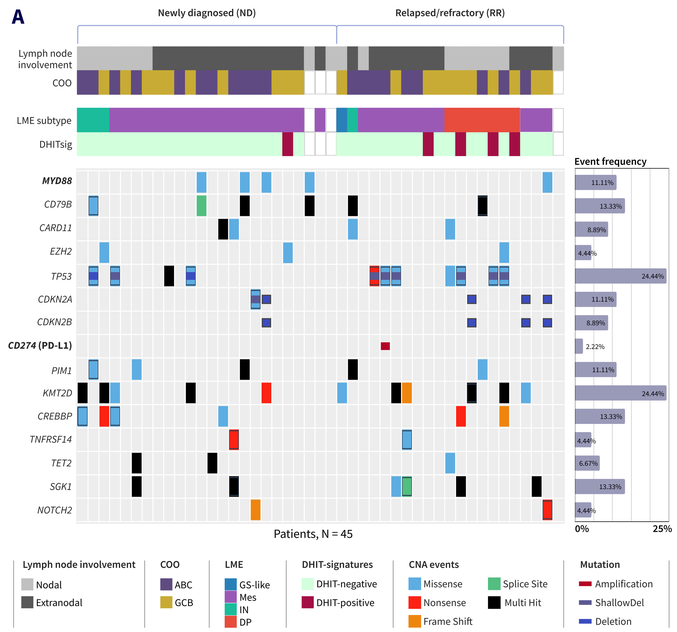

B

Metrics for clinically relevant outcomes

8 days	**73.3%**	**26.6%**
Median TAT	33 from 45 patients	12 from 45 patients
Median turnaround time (TAT) for Tumor Portrait™ test reports	Patients with WES/RNA-seq-measured biomarkers	Patients with matched clinical trials based on reported findings and patient location

Reported actionable events

RNA-seq	Cell of Origin (ABC) classification Predictive (*sensitivity to Ibrutinib-containing regimens; Lenalidomide ± Rituximab*); inclusion criterion for RCT.	13/45 (28.8%)
RNA-seq	DHITsig+ gene expression signature Prognostic (*inferior prognosis*)	6/45 (13.3%)
WES	*TP53* LOF mutation Prognostic (*inferior prognosis*)	13/45 (28.8%)
WES	*CDKN2A* gene loss Prognostic (*inferior prognosis*)	5/45 (11.1%)
WES	*CARD11* GOF mutation Predictive (*resistance to Ibrutinib*)	3/45 (6.7%)
WES	*EZH2* GOF mutation Predictive (*sensitivity to Tazemetostat*); inclusion criterion for RCT.	3/45 (6.7%)
WES	*MYD88*/*CD79B* co-mutation Predictive (*sensitivity to Ibrutinib*); inclusion criterion for RCT.	2/45 (4.4%)
WES	*CD274* (PD-L1) amplification Predictive (*sensitivity to Pembrolizumab*); inclusion criterion for RCT.	1/45 (2.2%)

Figure 2. A. Overview of reported clinical and molecular features. Investigational genomic findings and somatic variants of unknown significance are included in the plot. Results for cell of origin (COO) classification [1], lymphoma microenvironment (LME) subtypes [2], and double-hit expression signature (DHITsig) were not reported for patients with insufficient RNA quality. **B.** Summary of key clinically relevant outcomes and actionable events. RCT - recruiting clinical trials.

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Figure 3. Transcriptomic classifiers

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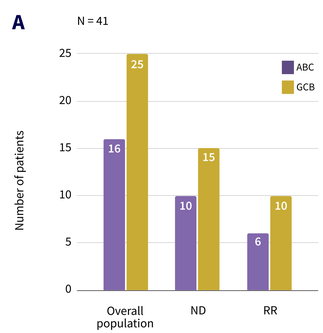

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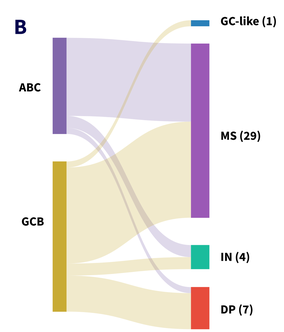

Germinal center-like (GC-like)
CD4⁺ T cells ↑
Follicular dendritic cells ↑
Lymphatic endothelial cells ↑

Mesenchymal (MS)
Cancer-associated fibroblasts ↑
Fibroblastic reticular cells ↑
Vascular endothelial cells ↑

Inflammatory (IN)
Neutrophils ↑
Macrophages ↑
CD8⁺ T-cells ↑

Depleted (DP)
Tumor proliferation ↑
SMAD1 methylation ↑

Figure 3. RNA-seq-based classifications. Number of patients for each measurement was limited by insufficient RNA quality and irrelevant diagnoses (COO was not reported for primary mediastinal large B-cell lymphoma).

A. Cell of origin (COO) subtyping with distribution of subtypes between newly-diagnosed (ND) and relapsed/refractory (RR) LBCL patients.

GCB — germinal center B-cell-like subtype; **ABC** — activated B-cell subtype.

B. Lymphoma microenvironment (LME) subtypes and distribution of the COO subtypes within the LME categories.

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Figure 4. LymphGen and LME classifiers

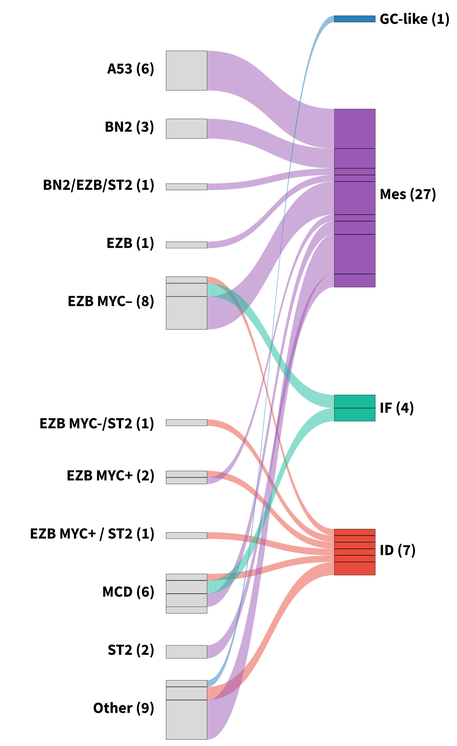

Figure 4. Genetic subtype distribution in LME categories.

Conclusions

Our results show the clinical utility and acceptable TAT of a comprehensive WES and RNA-seq assay on a cohort of lymphoma patients. The produced BostonGene Tumor Portrait™ test reports included findings on significant alterations, LymphGen and LME subtypes, COOs, and potential clinical trial matches. These robust findings, coupled with the rapid TAT, demonstrate the feasibility of using integrated WES and RNA-seq to guide clinical decision-making in lymphoma patients.

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