

## Transcriptional and genomic intra-tumor heterogeneity drives subclone specific drug responses in diffuse large B cell lymphoma [Abstract]

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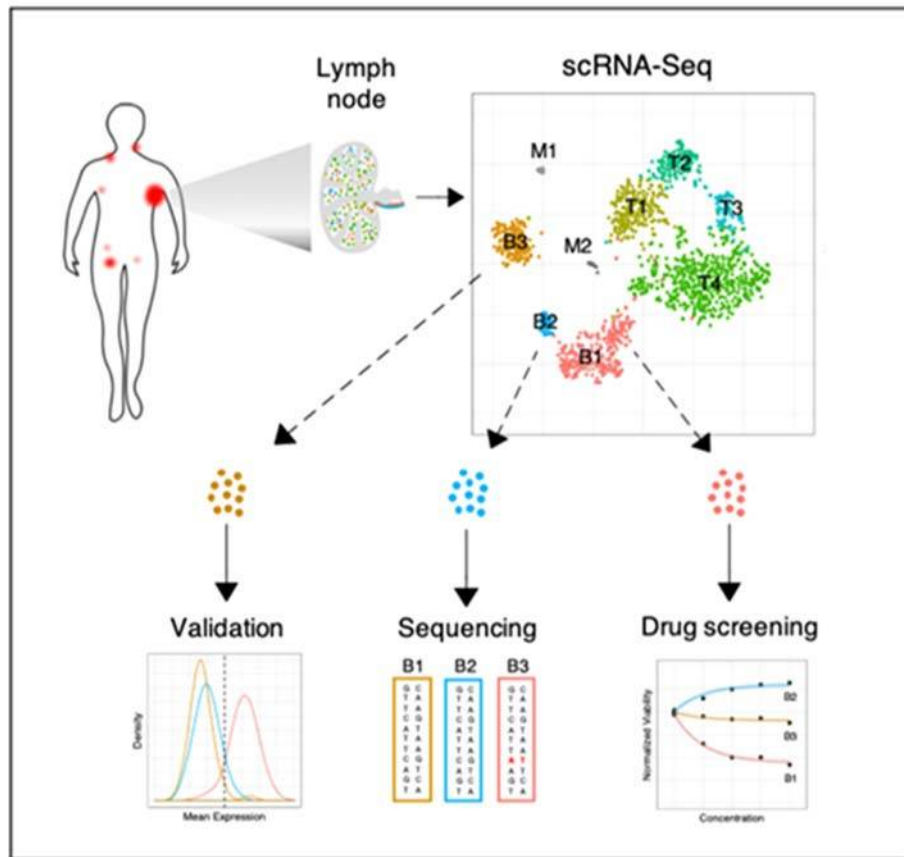


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**TRANSCRIPTIONAL AND GENOMIC INTRA-  
TUMOR HETEROGENEITY DRIVES  
SUBCLONE SPECIFIC DRUG RESPONSES IN  
DIFFUSE LARGE B CELL LYMPHOMA**

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**Figure 1. Study outline**



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**Introduction:** Little is known about intra-tumor heterogeneity of lymphoma and their complex disease-specific lymph node (LN) microenvironment.

**Methods:** To address this gap of knowledge we characterized follicular lymphoma, diffuse large B cell lymphoma (DLBCL) and reactive LN biopsies by comprehensive immunophenotyping (29 markers, n=40) and single cell RNA sequencing (scRNA-Seq, n=7). We used state-of-the-art clustering algorithms to identify malignant subclones and separated them by fluorescence-activated cell sorting (FACS). We further characterized these subclones by whole-genome sequencing (WGS) and extensive drug response profiling (64 drugs at 5 concentrations, Figure 1).

**Results:** We sequenced a total of 17,210 malignant B cells and 7,165 non-malignant bystander cells. Non-malignant cell subsets were

distinguished based on expression profiles of established marker genes, and malignant B cells were identified based on exclusive expression of either  $\kappa$  or  $\lambda$  light chain genes. Frequencies of B and T cells calculated based on scRNA-Seq highly correlated with frequencies of B and T cells calculated based on FACS analysis ( $r=0.97$ ) or immunohistochemistry analysis of corresponding paraffin sections ( $r=0.95$ ). These results suggested that our approach portrays a representative picture of each tumor and its microenvironment.

We further characterized non-malignant cell populations and identified, apart from well-described T cell subsets, a distinct T helper cell population with an exhaustion gene expression profile. Unsupervised clustering of all non-malignant cells from 7 LN biopsies grouped non-malignant cells by cell subtype rather than by sample origin. In contrast, frequencies of each T cell subset were highly variable across LN biopsies. These results suggested that expression profiles and functional states of T cell subsets were comparable across multiple LN, while each lymphoma conditions its own LN microenvironment by the abundance of different infiltrating T cells.

Next, we characterized malignant B cells of individual DLBCL patients and identified distinct subclones with unique gene expression profiles. We took advantage of differentially expressed surface markers (such as CD48, CD32, CD62L) and successfully sorted viable tumor cell subclones by FACS. We could demonstrate that lymphoma subclones of the same LN exhibited a strikingly different drug response profile. To understand differential drug responses, we performed WGS of each

subclone separately. A detailed comparison of gene mutation and copy number profiles between subclones of the same LN will be presented.

**Conclusions:** Together, our results uncover the complex cellular and clonal substructure of malignant B cell lymphomas. For the first time, our research links scRNA expression profiles of aggressive lymphoma subclones with distinct drug response, gene mutation and copy number profiles.

**Keywords:** diffuse large B-cell lymphoma (DLBCL); follicular lymphoma (FL); molecular genetics.