2859

Transcriptomic Comparison of Non-Hodgkin Lymphomas in Relapsed/Refractory Versus Newly Diagnosed Patients Using Single FFPE Slides

Omar Jabado[1], Christopher Chiu[1], Matthew Loya[1], Manling Ma-Edmonds[1], David Soong[2], Anantharaman Muthuswamy[1], Mahesh Bachu[1], Nicole Bottrel[3], Han Si[2], Jimin Zhang[1], Monica Wielgos-Bonvallet[1], Julie Blædel[4], Erin Henitz[3], Brandon Higgs[2], Kate Sasser[1], Suzana Couto[1], Maria Jure-Kunkel[1], Mark Fereshteh[1]

1 Translational Research, Genmab, Princeton, NJ; 2 Data Science, Genmab, Princeton, NJ; 3 Precision Medicine, Genmab, Princeton, NJ, 4 Medical Affairs, Genmab, Copenhagen, DK

OBJECTIVE

Identify genes, pathways, and immune contexture that distinguishes relapsed/refractory from newly diagnosed B-NHL tumors

CONCLUSIONS

The EdgeSeq platform enabled whole-transcriptome profiling from small-core needle biopsy samples in a lymphoma clinical trial

R/R DLBCL immune composition is reduced relative to newly diagnosed tumors and FL

R/R DLBCL tumor stroma was altered by angiogenesis and infiltration of fibroblasts

This study highlights the importance of characterizing the transcriptional heterogeneity across B-NHL stages in relation to T cell immunity and genetic alterations

References

1. Lenz G, Wright G, Dave SS, et al. *N Engl J Med.* 2008;359(22):2313-2323.
2. Ciavarella S, Vegliante MC, Fabbri M, et al. *Ann Oncol.* 2018;29(12):2363-2370.
3. McLean KC, Mandal M. *Trends Immunol.* 2020;41(7):629-642.
4. Cubedo E, Gentles AJ, Huang C, et al. *Blood.* 2012;119(23):5478-5491.
5. Parvin S, Ramirez-Labrada A, Aumann S, et al. *Cancer Cell.* 2019;36(3):237-249.e6.
6. Majdalawieh AF, Massri M, Ro HS. *J Oncol.* 2020;2020:8097872.
7. Zuo Y, Xu X, Chen M, Qi L. *Cancer Med.* 2021;10(13):4437-4450.
8. Fan H, Lu J, Guo Y, et al. *Nat Genet.* 2020;52(12):1384-1396.
9. Lan Q, Liu PY, Bell JL, et al. *Cancer Res.* 2021;81(13):3431-3440.
10. Aran D, Hu Z, Butte AJ. *Genome Biol.* 2017;18(1):220.
11. Ethiraj P, Sasi B, Holder KN, et al. *Br J Haematol.* 2022;198(2):349-359.
12. Monti S, Chapuy B, Takeyama K, et al. Cancer Cell. 2012;22(3):359-372.
13. Reddy A, Zhang J, Davis NS, et al. *Cell.* 2017;171(2):481-494.e15.
14. Jalali S, Ansell SM. *Leuk Lymphoma.* 2020;61(5):1028-1036.
15. Ennishi D, Jiang A, Boyle M, et al. *J Clin Oncol.* 2019;37(3):190-201.

Acknowledgments

We thank the patients who participated in the study.

We thank Alex Wolicki, Brad Forman and Angelo Harris from Genmab for sample management and histology support.

BACKGROUND

- Standard of Care (SoC) for patients with relapsed or refractory (R/R) B Cell Non-Hodgkin Lymphoma (B-NHL) has rapidly evolved over the past decade to incorporate chimeric antigen receptor T cell therapy (CAR T), immunomodulators, antibody-directed chemotherapies and bispecific antibodies.
- This diverse treatment landscape can affect the tumor microenvironment and drive the evolution of heterogenous escape mechanisms.
- Recent advances in the HTG EdgeSeq platform allow genome-scale profiling with minimal tissue; high multiplexing is achieved by deep sequencing on the Illumina platform.
- We successfully applied this technology to perform transcriptome-wide analysis from core needle samples in the EPCORE NHL-1 trial (NCT03625037).
- Ongoing analysis will uncover associations with International Prognostic Index score, Cell of Origin, effects of different treatment combinations, and genomic alterations on clinical outcomes.

MATERIALS AND METHODS

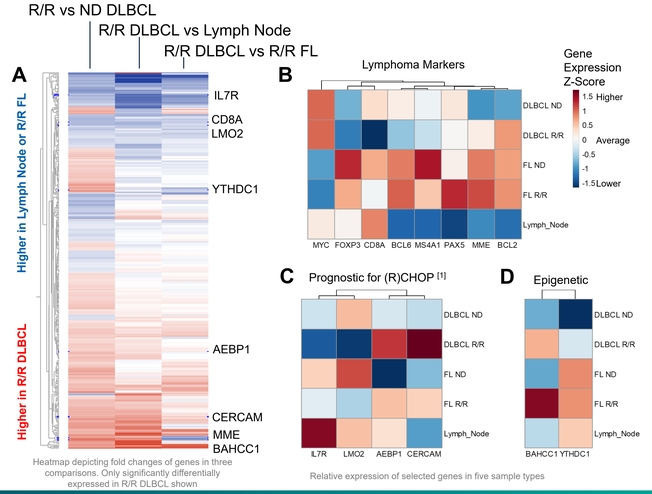
- Lysates were hybridized with HTG's Human Transcriptome Panel probes (19,000 genes).
- Average tissue input size was 8mm^2 for cores and 40mm^2 for resections with 5µm thickness.
- Formalin fixed, paraffin embedded (FFPE) samples from newly diagnosed (ND) patients with B-NHL and normal lymph node were sourced commercially. Baseline core needle biopsies from patients with relapsed/refractory (R/R) disease in NCT03625037 were obtained and processed with the commercially sourced samples.
- Patients in this study: R/R DLBCL 75, ND DLBCL 75, R/R FL 32, ND FL 19. Tumor locations included: lymphoid organs, gastrointestinal tract, testes, and the pleural cavity.
- Differential expression analysis and gene set enrichment was performed using linear models in R (Voom and LIMMA).
- Pathway gene sets were extracted from mSigDB (Hallmark) and Immune cell signatures were extracted from the literature (xCell[10]).

RESULTS

Substantial Changes in Relapsed/Refractory DLBCL Provide a Rich Dataset for Identification of Resistance Mechanisms

- Differential expression analysis identified 103 genes higher in R/R DLBCL and 80 genes higher in newly diagnosed (ND) DLBCL after multiple testing adjustment (**Figure 1A**).
- Comparison to lymph node controls can be used to identify genes that are associated with microenvironment changes, and comparison to R/R follicular lymphoma (FL) can reveal genes that represent common mechanisms of resistance.
- Well studied genes in lymphoma pathogenesis showed marked differences between R/R and ND patients (**Figure 1B**). *CD8A* was significantly depleted in whereas *BCL2, MYC* were enriched in R/R DLBCL. *FOXP3* was enriched in FL compared to DLBCL.
- The stromal context of DLBCL tumors is strongly prognostic for RCHOP therapy[1,2]; four example genes are illustrated in **Figure 1C**.
  - Down in R/R DLBCL: *IL7R*, a T cell and B cell regulator[3]; *LMO2,* a DNA repair sensor enriched in GCB[4] that sensitizes tumors to PARP inhibitors[5].
  - Up in R/R DLBCL: *AEBP1,* a *NF-κB* regulator[6] and *CERCAM* an endothelial adhesion molecule[7].
- Changes in epigenetic regulators were observed. *BAHCC1,* a histone methylation reader and oncogene[8] was enriched in R/R DLBCL and FL. In contrast, *YTHDC1*, involved in methyl RNA (m^{6}A) sensor was depleted in R/R and ND DLBCL (**Figure 1D**).

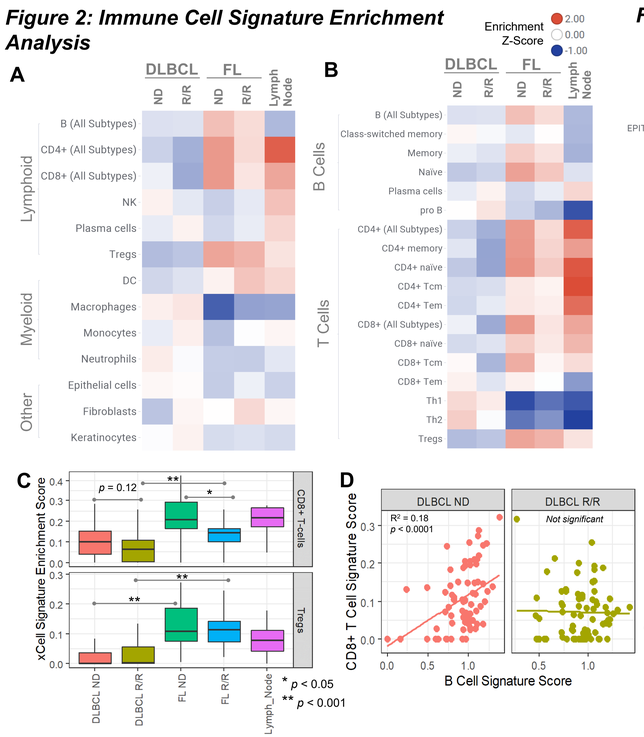
Figure 1. Genes Differentially Expressed in R/R DLBCL



The DLBCL Microenvironment has fewer T cells and is Remodeled with Mesenchymal Cells

- Immune-cell gene-signature analysis is a mature method that calculates proxies for cell-type abundance based on bulk RNA expression[10].
- DLBCL showed a marked decrease in T cell and DC signatures, and an increase of fibroblasts and other mesenchymal cell type signatures compared to FL (**Figure 2A, B**).

Figure 2: Immune Cell Signature Enrichment Analysis

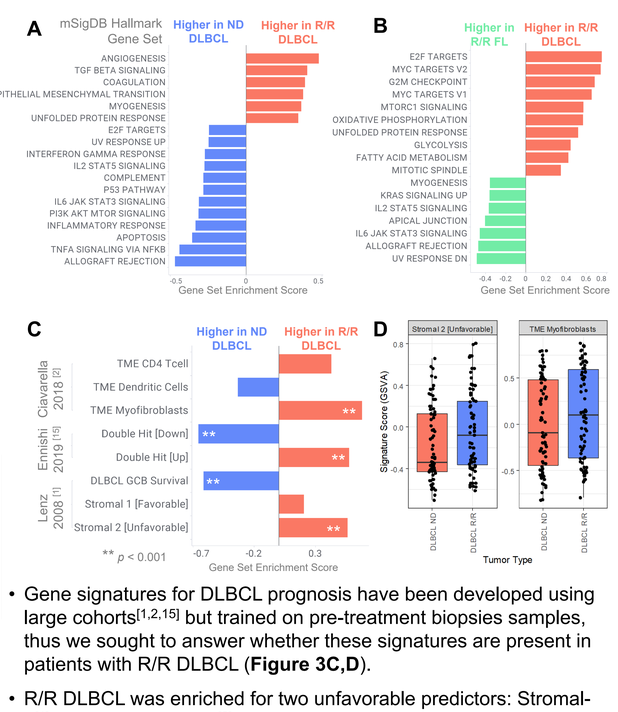


- CD8$^+$ T cell signature was depleted in R/R tumors in both DLBCL and FL; the T-reg signature was enriched in FL (**Figure 2C**).
- Interestingly, the CD8+ T cell signature was correlated to B cell signature (a proxy for tumor) in ND DLBCL but not R/R (**Figure 2D**).

Are Prognostic Signatures Derived from Tumors of ND Patients Relevant to R/R Patients?

- Angiogenesis and mesenchymal transition was enriched in R/R DLBCL; these processes have been posited as a mechanism of RCHOP resistance[1], potentially driven by *MYC*[11] (**Figure 3A**).
- *E2F, MYC* and glycolysis pathways were enriched in R/R DLBCL, versus R/R FL, consistent known oncogenes[12,13] and increased tumor metabolism[14] (**Figure 3B**).

Figure 3. Molecular Pathways and Prognostic Signatures



- Gene signatures for DLBCL prognosis have been developed using large cohorts[1,2,15] but trained on pre-treatment biopsies samples, thus we sought to answer whether these signatures are present in patients with R/R DLBCL (**Figure 3C,D**).
- R/R DLBCL was enriched for two unfavorable predictors: Stromal-2[1] and Myofibroblasts[2] both suggest mesenchymal remodeling.
- A "double-hit" signature for *MYC/BCL2* mutations in GCB[15] was also detected in R/R DLBCL, suggesting the presence of such patients in the cohort.

Presented at the 64th American Society of Hematology Annual Meeting and Exposition; December 10–13, 2022; New Orleans, LA, and virtual