SOHO ANNUAL MEETING 2024

A COMPREHENSIVE SINGLE-CELL MULTIOMIC ANALYSIS TO DEPICT THE BIOLOGICAL HETEROGENEITY OF FL AND DLBCL

S. HUERGA[1], B. ARICETA[2], P. AGUIRRE-RUIZ[2], P. SAN-MARTÍN[2], S. SARVIDÉ[2], A. LÓPEZ-JANEIRO[3], D. ALIGNANI[2], A. LÓPEZ-LÓPEZ[2], E. MUIÑOS-LÓPEZ[2], M. ABENGOZAR-MUELA[3], S. BROWNE[1], R. FIGUEROA[1], C. GRANDE[1], S. ROA[2], JR. RODRÍGUEZ-MADOZ[2], A. VILAS-ZORNOZA[2], F. PROSPER[1,2] and M. CANALES[1]

1. Hematology and Cell Therapy Department, Clínica Universidad de Navarra, Instituto de Investigaciones Sanitarias de Navarra (IdiSNA), Cancer Center Clínica Universidad de Navarra (CCUN) and Centro de Investigación Biomédica en Red de Cancer (CIBERONC), Pamplona, Spain
2. Hematology and Oncology Program, Centre for Applied Medical Research (CIMA), Instituto de Investigaciones Sanitarias de Navarra (IdiSNA), Cancer Center Clínica Universidad de Navarra (CCUN), Pamplona, Spain
3. Departament of Pathology, Clínica Universidad de Navarra, Pamplona, Spain

Cima Universidad de Navarra
Clínica Universidad de Navarra

INTRODUCTION

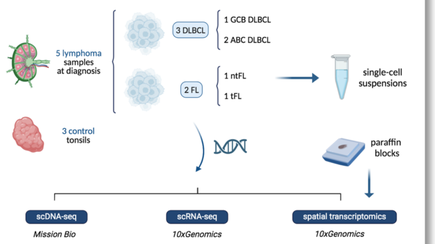
Diffuse large B cell and follicular lymphomas share a common germinal center B cell precursor. They originate from B cells at distinct stages of the germinal center reaction and through different interactions with the tumor microenvironment.

Also, 2% to 3% of follicular lymphoma patients transform into aggressive lymphoma each year, with no clear predictors.

AIM

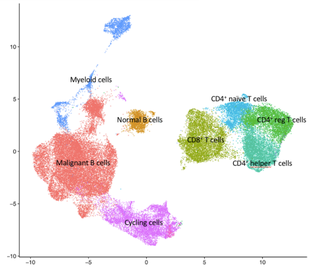
Using a multi-omic single-cell approach, we aimed to understand the molecular and transcriptional heterogeneity of both tumoral and TME cells in FL and DLBCL.

METHOD



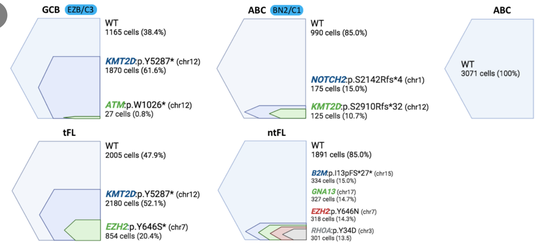
RESULTS

1



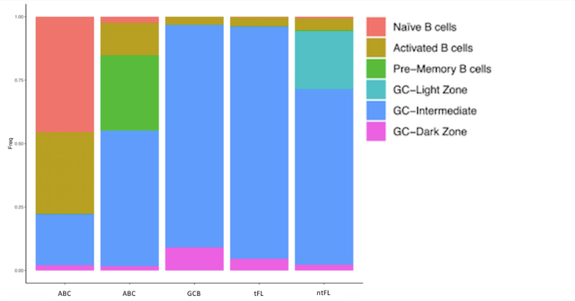
We identified 9 major clusters in the **scRNAseq** analysis. The most prevalent cells in the TME were T cells (CD4+).

4



Regarding **scDNAseq** analysis, no correlation between scRNA and scDNAseq clones. Molecular similarities between tFL and GCB.

2

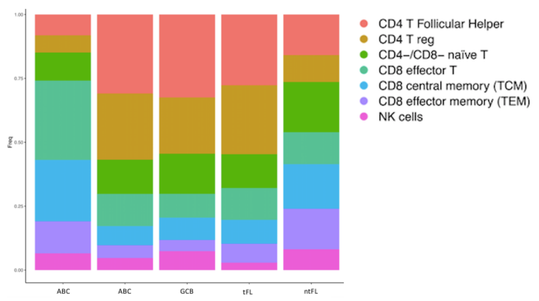


B cells were reclustered into 6 malignant and 3 non-malignant clusters. All samples contained subpopulations of several malignant clusters.

- DLBCL samples exhibited a high degree of heterogeneity. ABC may originate from pre-M or activated B cells.
- There were GC B cells in ABC patients.
- No differences between tFL and GCB DLBCL samples.

DZ	FoxO signaling pathway	e.g. *BTG1, GADD45A*
LZ	IFN-γ and immunoregulatory cytokines	e.g. *STAT1, IL15RA*
DZ/LZ	BCR activation	e.g. *KANK2, FOXP1*
Activated B	NF-kB pathway	e.g. *CD44, CD83*
Naïve B	Endocytosis and tumor angiogenesis genes	e.g. *SCARB2, FGF9*
Pre-M	B cell proliferation and immune regulation	e.g. *CTSZ, CD74*

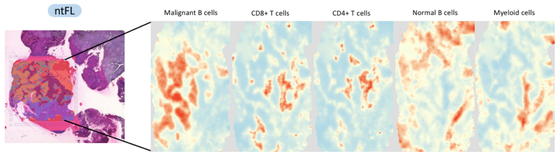
3



We identified **7 T-cell clusters**. DLBCL samples exhibited a high degree of heterogeneity. Again, there were no differences between tFL and GCB DLBCL samples.

CD4+ cells induced T cell tolerance (IL6/STAT3, PD-1), while exhaustion markers were highly expressed in effector CD8+. DLBCL and tFL had a stronger pattern of exhaustion. Follicular helper T cells in ntFL did not express exhaustion markers.

5



Finally, we performed **spatial transcriptomic analysis**. Completing it will allow us to further characterize the different microenvironments.

CONCLUSIONS

1) Our results confirm the heterogeneity of germinal center lymphomas and support the impact of the microenvironment in lymphomagenesis.
2) tFL patient was more similar to GCB than ntFL at diagnosis. Some potential predictors of FL transformation at diagnosis could be: high levels of CD4$^+$, low levels of naïve T and myeloid cells, exhausted follicular helper T cells and a DZ/LZ transcriptional pattern.
3) There was no clear correlation between scDNA and scRNAseq subclones. Our samples appear to follow a linear evolution model. Epigenetic changes, such as *KMT2D* mutations, were early events, suggesting that some epigenetic drugs could be effective.

REFERENCES

1. Holmes AB, et al. Single-cell analysis of germinal center B cells informs on lymphoma cell of origin and outcome. *J Exp Med*. 2020; 217 (10) : e20200483
2. Haebe S, et al. Single-cell analysis can define distinct evolution of tumor sites in follicular lymphoma. *Blood*. 2021;137(21):2869-2880
3. Ye X, et al. A single-cell atlas of diffuse large B cell lymphoma. *Cell Rep*. 2022;39(3):110713

CONTACT INFORMATION

Sofía Huerga Domínguez, MD

Clínica Universidad de Navarra, Hematology and Cell Therapy Department
Avenida Pío XII, 36, 31008, Pamplona (Spain)

Email: shuergad@unav.es