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POSTER ABSTRACTS

621.LYMPHOMAS: TRANSLATIONAL-MOLECULAR AND GENETIC

A Multi-Omic Study Unveils a Clonal Population of TET2-Mutated Infiltrating T-Cells in Germinal Center B Cell Lymphomas (DLBCL and FL)

Sofia Huerga[1], *Beñat Ariceta*[2], *Paula Aguirre-Ruiz*[3], *Patxi San-Martin*[2], *Sarai Sarvide*[2], *Marta Abengozar-Muela, MD*[4], *Diego Alignani*[2], *Rocio Figueroa, MD PhD*[1], *Carlos Grande, MD PhD*[1], *Alvaro Lopez-Janeiro, MD*[5], *Emma Muiños-Lopez, PhD*[3], *Juan Roberto Rodriguez-Madoz, PhD*[2], *Amaia Vilas-Zornoza*[2], *Felipe Prosper, MDPhD*[1], *Miguel Canales*[6]

[1] Hematology and Cell Therapy Department, Clínica Universidad de Navarra, Instituto de Investigaciones Sanitarias de Navarra (IdiSNA), Cancer Center Clinica 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 Clinica Universidad de Navarra (CCUN), Pamplona, Spain
[3] Hematology and Oncology Program, Centre for Applied Medical Research (CIMA), Instituto de Investigaciones Sanitarias de Navarra (IdiSNA), Cancer Center Clinica Universidad de Navarra (CCUN), Pamplona, Spain, Pamplona, Spain
[4] Department of Pathology, Clinica Universidad de Navarra (CUN), Pamplona, Spain
[5] Departament of Pathology, Clínica Universidad de Navarra, Pamplona, Spain
[6] Clinica Universidad de Navarra Cancer Center (CCUN), Pamplona, Spain

Introduction

Follicular lymphomas (FL) and diffuse large B cell lymphomas (DLBCL) share a common germinal center (GC) B cell precursor. Nevertheless, these lymphomas appear to originate from different developmental stages, through distinct pathogenetic mechanisms and their clinical behavior varies significantly, even among FL or DLBCL patients. To understand the molecular and transcriptional heterogeneity underlying this clinical diversity we conducted a multi-omic study in GC B cell lymphomas.

Methods

Our study comprised 3 DLBCL patients (1 non-GCB (germinal center B), 1 non-GCB CD5+, and 1 GCB) and 2 FL (1 with no transformation (ntFL) and 1 patient with transformation (tFL)) at diagnosis. We performed single-cell multiome-sequencing (scDNA-seq + scProtein) (*Tapestri platform* -Mission Bio-) and single cell RNA-sequencing (scRNA-seq) (*Chromium system* -10xGenomics-) from cell suspensions and spatial transcriptomics (*Visium technology* -10xGenomics-) from paraffin blocks.

Results

Genotyping data were available for 19,700 cells. A total of 311 variants across the 5 samples passed all quality controls. We selected probably pathogenic and pathogenic variants for downstream analysis. Germline variants were discarded.

In 3 of 5 patients, we detected 1 somatic and nonsynonymous variant defining the first clone (variants in *KMT2D* and *NOTCH2* mutated in 6-38% of the cells) and another somatic and nonsynonymous variant, defining a subclone (variants in *KMT2D*, *ATM*, *EZH2* mutated in 1-15% of the cells). In 1 of 5 patients (ntFL), we detected up to 4 mutations, acquired linearly (*B2M*, *GNA13*, *EZH2*, *RHOA* mutated clone represents 8% of the cells). Remarkably, all patients showed the same pathogenic variant in *TET2* (1-2% of the cells), as an independent clone. The highest molecular similarity was observed between the GCB DLBCL patient and the tFL patient. Both had the same variant in *KMT2D* as the first hit (later GCB-DLBCL acquired *ATM*, while tFL acquired *EZH2* as a second hit). *ATM* and *EZH2* mutations have been described in "bulk" studies conducted on DLBCL and FL patients, respectively.

Regarding protein expression, there was a good correlation between GCB and non-GCB phenotypes studied by immunohistochemistry and scProtein (the expression of CD10 by proteomics was lower in non-GCB patients). *TET2* clones described by scDNA-seq were enriched in CD5+ and CD19- cells, suggesting they belong to the T-lymphoid lineage. The other clones and subclones were enriched in CD19+ and CD5- cells (*Figure 1*).

Data from 54,024 single cells were obtained for scRNA-seq analysis, with an average of 10,805 cells (7,335-18,205) for each sample. Cell type annotation was performed based on the expression of canonical markers. We identified 13 different clusters,

the main ones being: GC B cells, post-GC B cells, naïve B cells, myeloid cells, CD8+ cytotoxic T cells, CD4+ naïve T cells, CD4+ reg T cells, CD4+ helper T cells.
All samples contained subpopulations of both GC and post-GC B cell clusters. However, GCB DLBCL and FL patients were significantly enriched in GC B cells (63.2 vs 22.2%; $p<0.01$), whereas non-GCB patients were significantly enriched in post-GC B cells (35.5 vs 2.9%; $p<0.01$). Part of the normal B-cell transcriptional function was preserved in all samples (*Figure 2*). The analysis of the infiltrating T cells revealed that the CD4+/CD8+ T cells ratio progressively increased from ntFL (1.14) to tFL (2.25) and DLBCL (2.58). Furthermore, we estimated the exhaustion status of T cells by the expression of these five markers (*TIGIT, LAG3, CTLA4, HAVCR2, PDCD1*). Exhaustion T cell markers were highly expressed in CD8+ cytotoxic T cells and cycling T cells and lower expressed in CD4+ reg T cells. Both DLBCL and tFL have a stronger pattern of exhaustion than ntFL.
Finally, by the spatial transcriptomics analysis, we have identified distinct cell type proportions according to their matched single-cell RNA data.

Conclusions

Our results confirm the intra and intertumor heterogeneity in GC B cell lymphomas. Notably, the greatest molecular and transcriptional similarities are observed between the GCB DLBCL and tFL patients at diagnosis. Moreover, our findings support the critical role of the tumor microenvironment for the persistence and development of the tumor clone. The finding of a *TET2* mutated clone in the infiltrating T cells of all the patients is particularly noteworthy, as it may suggest the possible presence of clonal lymphopoiesis.

Disclosures Grande: *AbbVie:* Other: Advisory Board. **Canales:** *Beigene:* Consultancy; *BMS:* Consultancy; *Incyte:* Consultancy; *Janssen:* Consultancy; *Karyopharm:* Consultancy; *Kite:* Consultancy; *Kyowa:* Consultancy; *Lilly:* Consultancy; *Roche:* Consultancy; *Takeda:* Consultancy; *Incyte:* Speakers Bureau; *Janssen:* Speakers Bureau; *Kite:* Speakers Bureau; *Kyowa:* Speakers Bureau; *Roche:* Speakers Bureau; *Takeda:* Speakers Bureau.

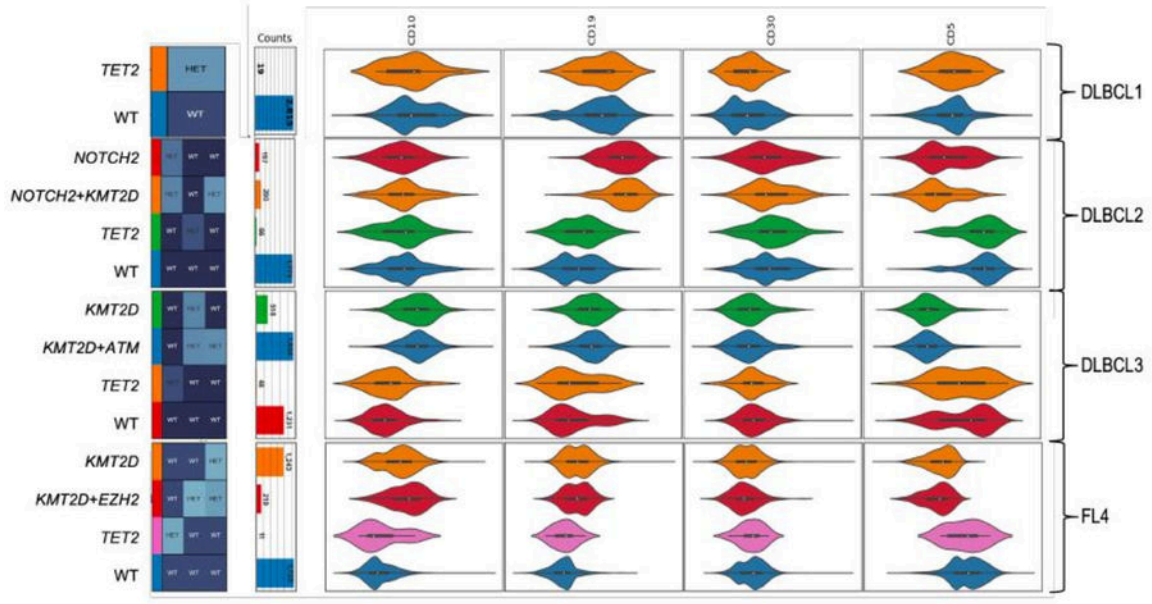

Figure 1. ScProtein: protein expression of different clones described by single cell DNA-sequencing (scDNA-seq).
DLBCL: diffuse large B cell lymphomas; FL: follicular lymphomas; WT: wildtype.

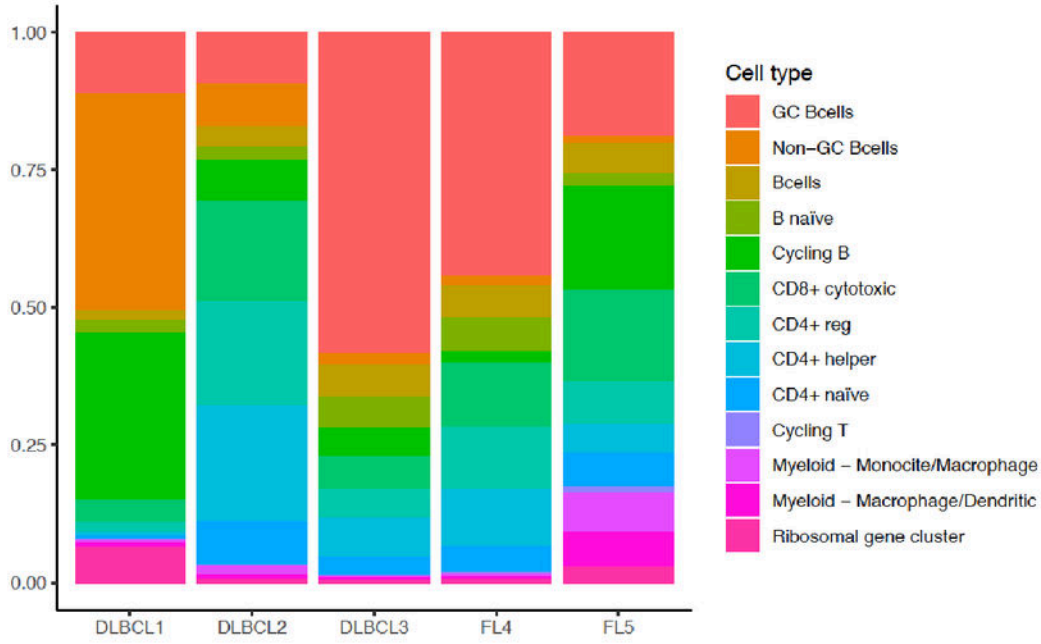

Figure 2. ScRNA-seq (single cell RNA-sequencing) data: relative proportions of tumor and tumor microenvironment subclones for each sample. In total, 31.060 B cells (57.5%), 18.790 T cells (34.8%), and 4174 myeloid cells (7.7%) were analyzed.
GC: germinal center

Figure 1

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