Poster #: 1021

XXI IWCLL International Workshop on CLL

Normal B Cells in MBL Show Abnormal Transcriptomic and Subset Distributions: Evidence for Follicular Maturation in IGHV-Mutated MBL and Extrafollicular Maturation in IGHV-Unmutated MBL

Gonzalo Blanco[1], Joy Yan[1], Anita Ng[1], Andrew Shih[2], Jacqueline Aldridge[3], Anna Puiggros[4], Lindybeth Sarmiento[3], Jonathan Kolitz[5], Steven Allen[5], Kanti Rai[5], Douglas Stewart[6], Blanca Espinet[4], Gerald Marti[6], Neil Caporaso[6], Marcelo Navarrete[3], Nicholas Chiorazzi[1]

[1]*Karches Center for Oncology Research, The Feinstein Institutes for Medical Research, Manhasset, US*
[2]*Robert S. Boas Center for Genomics and Human Genetics, The Feinstein Institutes for Medical Research, Manhasset, US*
[3]*Universidad de Magallanes, Punta Arenas, Chile*
[4]*Translational Research on Hematological Neoplasms Group, Hospital del Mar Medical Research Institute, Barcelona, Spain*
[5]*Department of Medicine, North Shore University Hospital and LIJ Medical Center, Northwell Health, Manhasset, US*
[6]*National Institutes of Health, Bethesda, US*

OBJECTIVES: To characterize **transcriptomic profiles**, **B-cell subset distributions**, and **BCR repertoires** of normal B cells (**NBC**) from people with **MBL** and healthy donors (**HD**).

CONCLUSIONS

- Our results demonstrate a **global B-cell defect both in lcMBL and hcMBL, being markedly more pronounced in the latter.** The transcriptomic differences (NBC from lcMBL being much closer to HD), together with the different immunogenetic pressures revealed by BCR analysis point to separate pathogenic drivers in lcMBL and hcMBL.
- **In hcMBL, the transcriptomic defects were demonstrated in CD5$^+$ and CD5$^-$ NBC subsets and in M-MBL and U-MBL individuals**.
- **NBC from M-hcMBL appear to follow a follicular B-cell maturation pathway**, marked by unique BER and MMR gene signatures, enriched switched memory and DN1 B cells, and a more restricted and hypermutated BCR repertoire.
- Conversely, **NBC from U-hcMBL** show a gene signature of ongoing inflammation compatible with **extrafollicular B-cell maturation**, supported by an enrichment in unswitched memory and DN2/3 B-cell subsets and reduced SHM rates.
- These findings suggest that **different antigenic challenges push hcMBL NBC into distinct developmental paths that correlate with IGHV-mutation status and that differ from lcMBL**. Whether the latter represents an **MBL-specific process** or an indicator of developmental differences within the lcMBL group **needs to be determined**.

Feinstein Institutes for Medical Research
Northwell Health®

CONTACT

Gonzalo Blanco: gblancoares@northwell.edu

Nicholas Chiorazzi: nchizzi@northwell.edu

INTRODUCTION

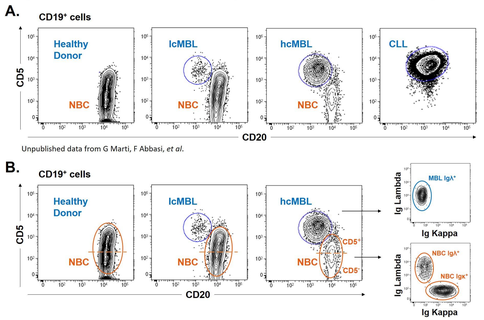
- **CLL** and its precursor, **monoclonal B-cell lymphocytosis** (**MBL**), are associated with **immune deficits**
- In MBL, the higher percentages of **normal B cells** (**NBC**) provide a unique opportunity to **study B-cell defects** (Figure 1A)

METHODS

A. RNA-seq of sorted B-cell fractions:

- We evaluated the **transcriptomic variance of NBC** from:
  - 21 hcMBL (12 M-MBL, 9 U-MBL)
  - 7 lcMBL (1 M-MBL, the rest unknown)
  - 13 age-matched HD
- In all, **111 FACS-sorted B-cell samples** were obtained (Figure 1B):
  - 25 NBC-HD **CD20$^+$CD5$^-$**Igκ$^+$/Igλ$^+$
  - 17 NBC-HD **CD20$^+$CD5$^+$**Igκ$^+$/Igλ$^+$
  - 12 NBC-lcMBL **CD20$^+$CD5$^-$**Igκ$^+$/Igλ$^+$
  - 30 NBC-hcMBL **CD20$^+$CD5$^-$**Igκ$^+$/Igλ$^+$
  - 6 NBC-hcMBL **CD20$^+$CD5$^+$**Igκ$^+$/Igλ$^+$
  - 21 hcMBL clones **CD20^{Low}CD5$^+$**Igκ$^+$/Igλ$^+$

Figure 1. RNA-seq study rationale and design



A. The percentage of normal B cells (NBC) decreases as the tumor B-cell clone expands. B. FACS-sorting strategy employed.

- RNA was sequenced using **SMART-Seq v4** on a **HiSeq** platform
- **Differentially expressed genes** (**DEG**) were analyzed with DESeq2 (**Padj<0.05**, **|FC|≥1.5**)
- Transcriptome variation was assessed by **Principal Component Analysis (PCA)** and enriched pathways by **Ingenuity Pathway Analysis (IPA)**

B. Flow cytometry analysis of B-cell subsets:

- **B-cell subset** distribution was analyzed on **CD5$^-$ NBC** from:
  - 14 hcMBL (10 M-MBL, 4 U-MBL)
  - 9 age-matched HD
- **FACSymphony** and **FlowJo** were employed

C. BCR repertoire analysis:

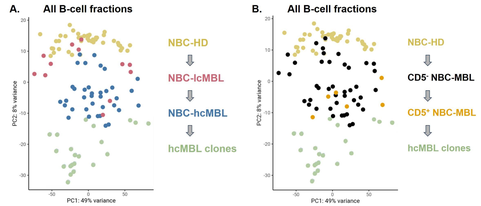
- **BCR repertoire** was performed on **CD5$^-$ NBC** from:
  - 7 hcMBL (4 M-MBL, 3 U-MBL)
  - 1 M-lcMBL
  - 4 age-matched HD
- The **ImmunoRead** platform and a custom pipeline built upon the **Immcantation framework** were used

RESULTS

A.1. RNA-seq of all FACS-sorted B-cell fractions:

- **PCA clearly separated** NBC-HD, NBC-lcMBL, NBC-hcMBL, and hcMBL clones, **forming a gradient** with **NBC-HD** and **hcMBL clones** at **opposite extremes**, and NBC-lcMBL samples occupying an intermediate position between NBC-HD and NBC-hcMBL (Figure 2A)
- **CD5$^+$ NBC-MBL** clustered **closer to hcMBL clones** (Figure 2B)

Figure 2. Principal Component Analysis (PCA) of all FACS-sorted B-cell fractions

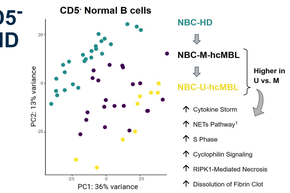


A. NBC categorized according to diagnosis (HD, lcMBL, hcMBL). B. NBC from MBL patients categorized according to CD5 positivity.

A.2. RNA-seq of CD5$^-$ NBC from hcMBL and HD:

- **PCA separated** CD5$^-$ NBC from M-hcMBL and U-hcMBL into **two distinct clusters,** with the **U-hcMBL** group positioned **farther from the NBC of HD** (Figure 3)
- **Comparing CD5$^-$NBC-M-hcMBL to NBC-HD revealed:**
  - 8,380 DEG
  - **Most IPA pathways**, including B-cell activation (20/21, 95%), **inhibited**
  - Genes involved in mismatch and base-excision repair (**MMR, BER**), typically found in **follicular B-cell responses**, were **activated**
- **Comparing CD5$^-$NBC-U-hcMBL to NBC-HD showed:**
  - 12,793 DEG
  - Widespread **upregulation of activation pathways** (23/26, 88%), including inflammatory networks
- **CD5-NBC-U-hcMBL vs. NBC-M-hcMBL disclosed:**
  - Fewer DEG (1,316), since both groups are hcMBL
  - **U-MBL** showed **activated pathways** (cytokine storm, NET, S-phase and cyclophilin signaling, and RIPK1-mediated necrosis, Figure 3), typical for **heightened inflammation** and **extrafollicular B-cell responses**

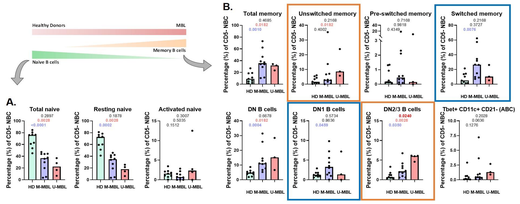
Figure 3. PCA of CD5$^-$ NBC from hcMBL and HD



B. Flow cytometry analysis of B-cell subsets:

- Phenotypically, **NBC-hcMBL** had **reduced naïve B cells** (Figure 4A), and **increased memory, ABC and DN B cells** (Figure 4B) compared to NBC-HD (P<0.05)
- **NBC-M-hcMBL** exhibited **increased DN1** cells (P=0.046), considered switched memory precursors, and **expanded switched memory B cells** (P=0.008)
- **NBC-U-hcMBL** displayed **increased unswitched memory B cells** (P=0.018) and **higher DN2/3** cells (precursors to antibody-secreting cells) compared to NBC-HD (P=0.003) and NBC-M-hcMBL (P=0.024)

Figure 4. Flow cytometry analysis of B-cell subsets

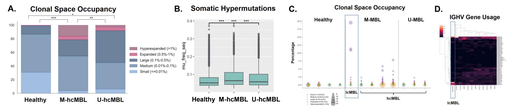


A. Naïve B-cell subsets. B. Memory B-cell subsets. Significant P-values are bolded; blue indicates significant differences between CD5$^-$ NBC of M-MBL vs. HD; red indicates significant differences between CD5$^-$ NBC of U-MBL vs. HD; maroon indicates significant differences between CD5$^-$ NBC of U-MBL vs. M-MBL. Differences unique to NBC in the M-MBL group are outlined in blue boxes, whereas those unique to U-MBL are outlined in orange boxes. DN: double negative B cells, ABC: age-associated B-cells.

C. BCR repertoire analysis:

- **NBC-HD** exhibited a **polyclonal** repertoire; **NBC-U-hcMBL** showed **increased expanded and hyperexpanded clones** (5% each); and **NBC-M-hcMBL had the most restricted repertoire**, with 16.3% hyperexpanded clones (Figure 5A)
- **NBC-M-hcMBL** also displayed **the highest SHM rates**, surpassing NBC-U-hcMBL and NBC-HD (P<0.001, Figure 5B)
- The sole **M-lcMBL** case showed a **clearly distinct BCR repertoire**, with a high proportion of hyperexpanded clones (Figure 5C) and unique V gene usage (Figure 5D)

Figure 5. BCR repertoire analysis



For panels A and B, only hcMBL patients are included. C and D include and highlight the single lcMBL patient.

REFERENCES

1. Bukhari, A., et al. Death of tonsillar B cells by NETosis. *Cell Death Discov.* 9, 108 (2023)

ACKNOWLEDGMENTS

GB was supported by the Lymphoma Research Foundation

DISCLOSURES

Nothing to disclose