XIX CONGRESSO NAZIONALE SIES 2026

SPATIAL TRANSCRIPTOMIC ANALYSIS OF CHRONIC LYMPHOCYTIC LEUKEMIA LYMPH NODES REVEALS TUMOR MICROENVIRONMENT EVOLUTION DURING DISEASE PROGRESSION

G. Notarpietro[1], A. Bucci[1], M. C. Vegliante[1], B. Apollonio[2], S. A. Pappagallo[1], P. Mondelli[1], R. Daprile[3], A. Scattone[3], M. Frenquelli[4], A. Guarini[1], G. Castellano[5], A. Ciarrocchi[6], B. Donati[6], M. Ponzoni[7], P. Ghia[8], S. Ciavarella[1] , C. Minoia[1]
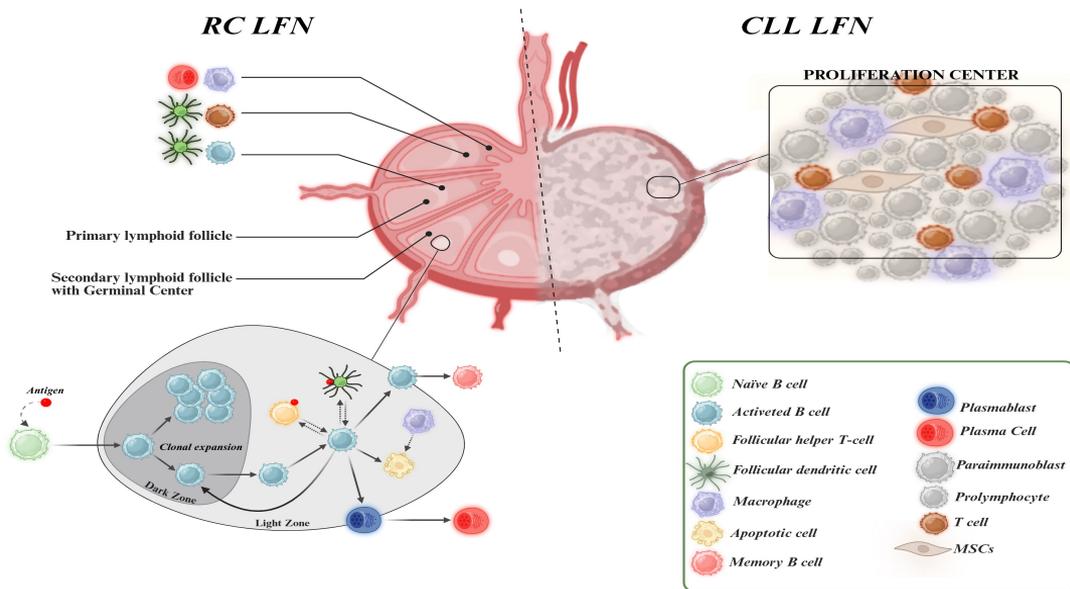
[1]IRCCS Istituto Tumori "Giovanni Paolo II", Hematology Unit; [2]IRCCS Istituto Tumori "Giovanni Paolo II", Rare Tumors and Melanoma Unit; [3]IRCCS Istituto Tumori "Giovanni Paolo II", Pathology Unit; [4]Comprehensive Cancer Center, IRCCS Ospedale San Raffaele, B-cell Neoplasia Unit Division of Experimental Oncology; [5] Sant Joan de Déu; [6]Laboratory of Translational Research, Azienda Unità Sanitaria Locale-IRCCS of Reggio Emilia; [7]Pathology Unit, IRCCS Ospedale San Raffaele; [7,8] Università Vita-Salute San Raffaele

Firenze | 4-6 marzo 2026
Palazzo degli Affari

Disclosures of Giulia Notarpietro (none)

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other

CLL: TME dynamics into Lymph Node compartments

RC LFN

CLL LFN

PROLIFERATION CENTER

Primary lymphoid follicle

Secondary lymphoid follicle with Germinal Center

Antigen

Clonal expansion

Dark Zone

Light Zone

Legend:
- Naïve B cell
- Activeted B cell
- Follicular helper T-cell
- Follicular dendritic cell
- Macrophage
- Apoptotic cell
- Memory B cell
- Plasmablast
- Plasma Cell
- Paraimmunoblast
- Prolymphocyte
- T cell
- MSCs

- **Mature B-cell neoplasia**: accumulation in PB, BM, secondary lymphoid organs
- **Dynamic kinetics:** quiescent in PB → proliferative in PCs (80-90% LN biopsies)
- **Lymph node TME:** key driver of pathogenesis/progression
- **CLL-TME dependency:** survival/proliferation via NLCs, T cells, MSCs
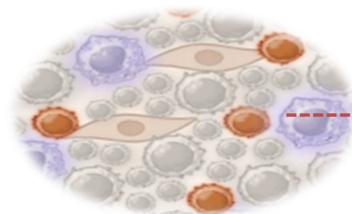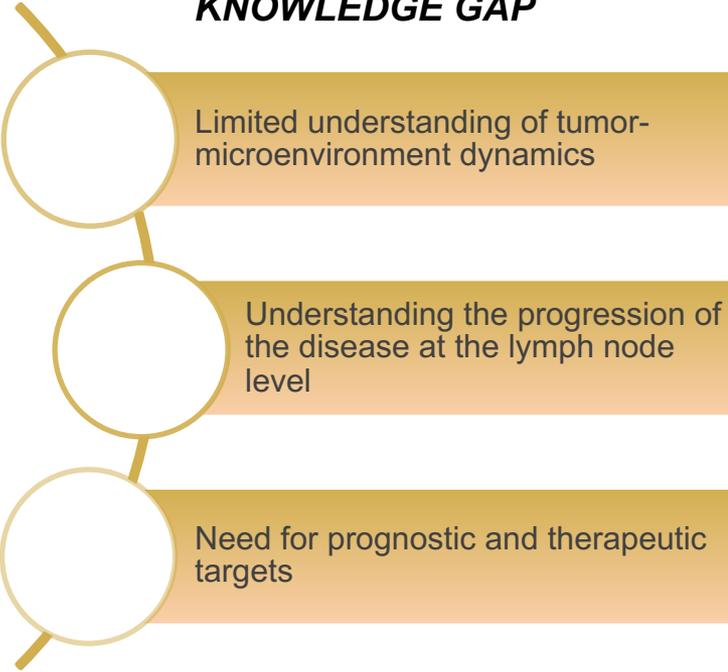
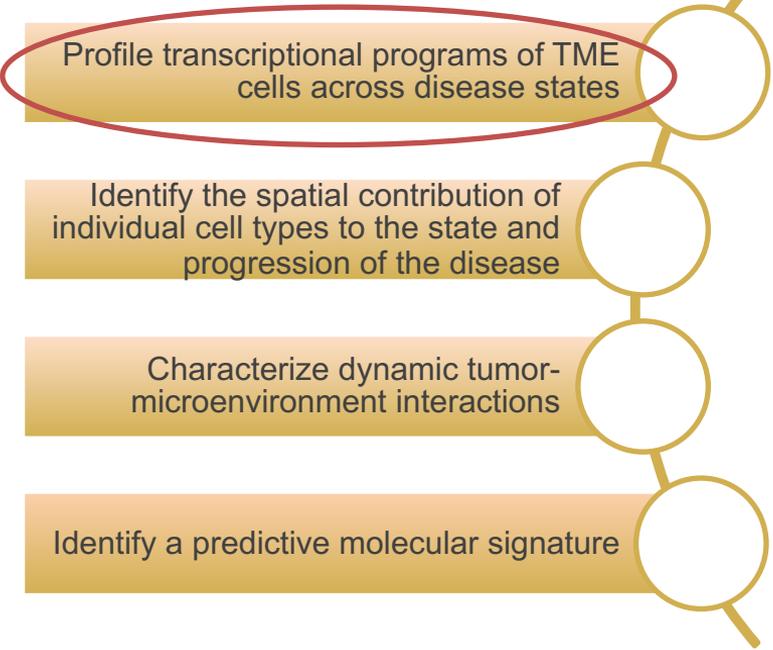**How does TME evolve and co-evolve through disease progression?**

Ponzoni et al. (2011) ; Bucci, Notarpietro et al. submitted

Unveiling the contribution of microenvironmental components in the progression of CLL

KNOWLEDGE GAP

- Limited understanding of tumor-microenvironment dynamics

- Understanding the progression of the disease at the lymph node level

- Need for prognostic and therapeutic targets

STUDY OBJECTIVES

- Profile transcriptional programs of TME cells across disease states

- Identify the spatial contribution of individual cell types to the state and progression of the disease

- Characterize dynamic tumor-microenvironment interactions

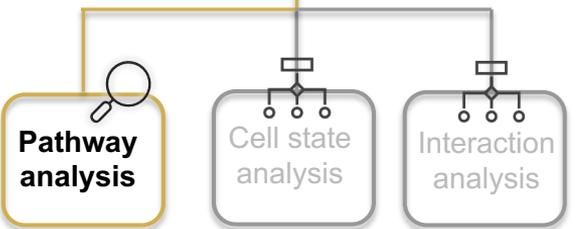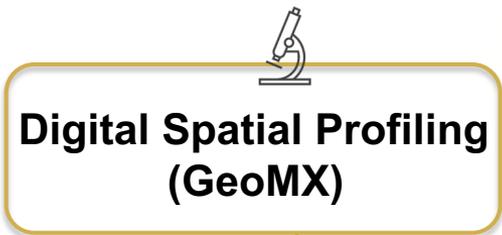- Identify a predictive molecular signature

Study Design and Workflow

Discovery phase

17 FFPE LN (IRCCS Bari)

- **A** (n=6) indolent CLL
- **B** (n=4) CLL requiring treatment within 6 months
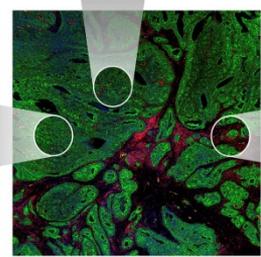- **C** (n=7) relapsed/refractory CLL

Digital Spatial Profiling (GeoMX)

Pathway analysis

Cell state analysis

Interaction analysis

Validation phase

Signature selection

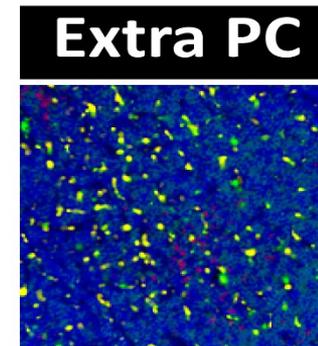**Spatial Profiling**
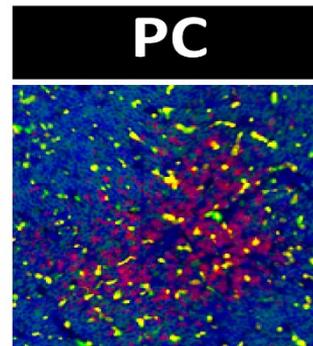Differential Expression between tumor/TME

Pathway Analysis

Biomarker Discovery

Study Design and Workflow

NUCLEI MUM1 CD3 CD68

Proliferation center (PC)

Extra-PC

PC
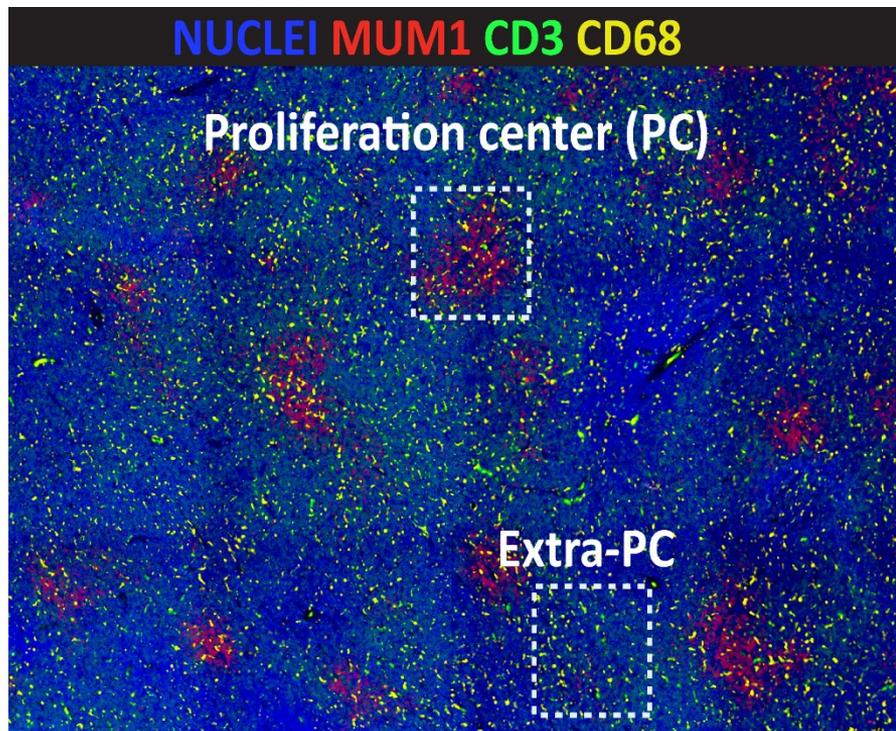
Extra PC

Tot. 284 ROIs					
PC (MUM$^+$)			Extra PC (MUM$^-$)		
CD3$^+$	CD68$^+$	CLL	CD3$^+$	CD68$^+$	CLL
38	38	44	50	57	57

PC – **CD68+** macrophages undergo transcriptional reprogramming during CLL progression

GSEA – CD68 PC (A vs B)

DEGs: 159

- Cell Adhesion
- Enzyme Linked Receptor Protein Signaling Pathway
- Cell Activation
- Cell Cell junction
- DNA Binding Transcription Factor Activity

DEGs: 373

- DNA Metabolic Process
- Oxidoreductase Activity
- Nucleobase Containing Small Molecule Metabolic Process
- Mitochondrial Gene Expression
- Mitochondrial Translation

Normalized Enrichment Score (NES)

GSEA – CD68 PC (B vs C)

DEGs: 137

- Nucleobase Containing Small Molecule Metabolic Process
- Purine Containing Compound Metabolic Process
- Monocarboxylic Acid Metabolic Process
- mRNA Metabolic Process
- Mitochondrial Envelope

DEGs: 408

- Transporter Activity
- Cytoskeletal Protein Binding
- Passive Transmembrane Transporter Activity
- Positive Regulation Of Programmed Cell Death
- Cell Projection Organization

Normalized Enrichment Score (NES)

- **Group A (Indolent):** ↑ Cell activation/adhesion, trascriptional activity
- **Group B (Need of treatment):** Metabolic activity, mitochondrial translation
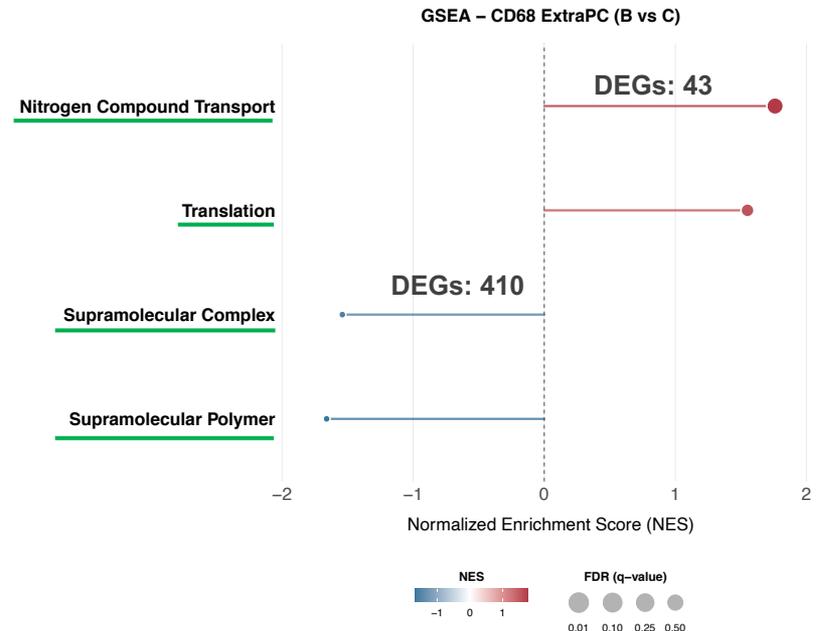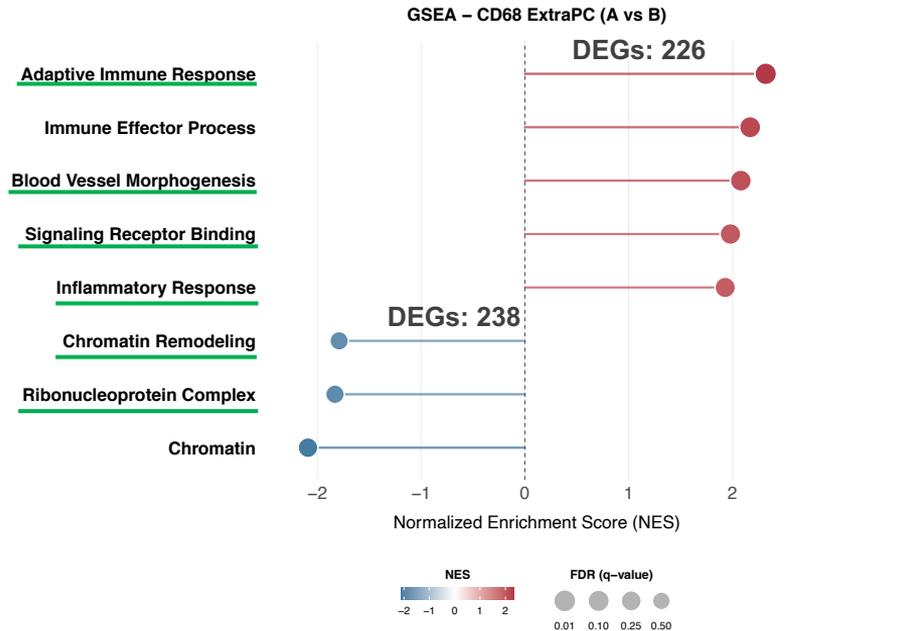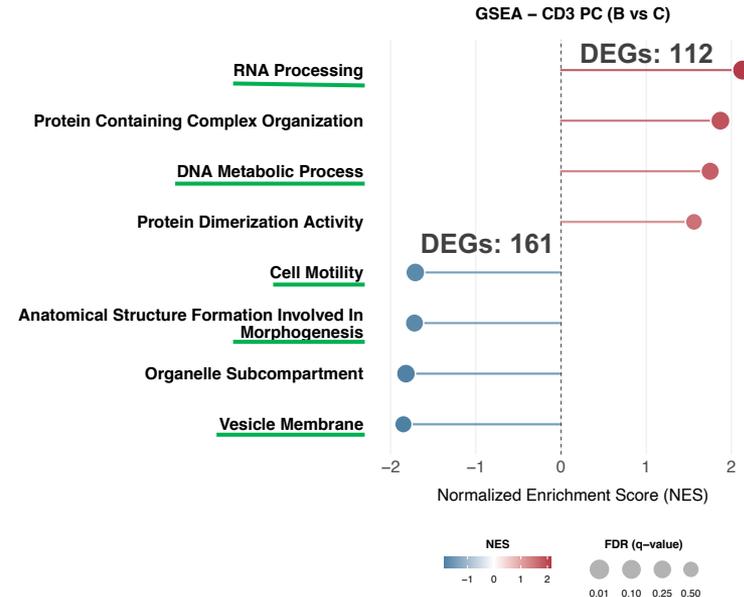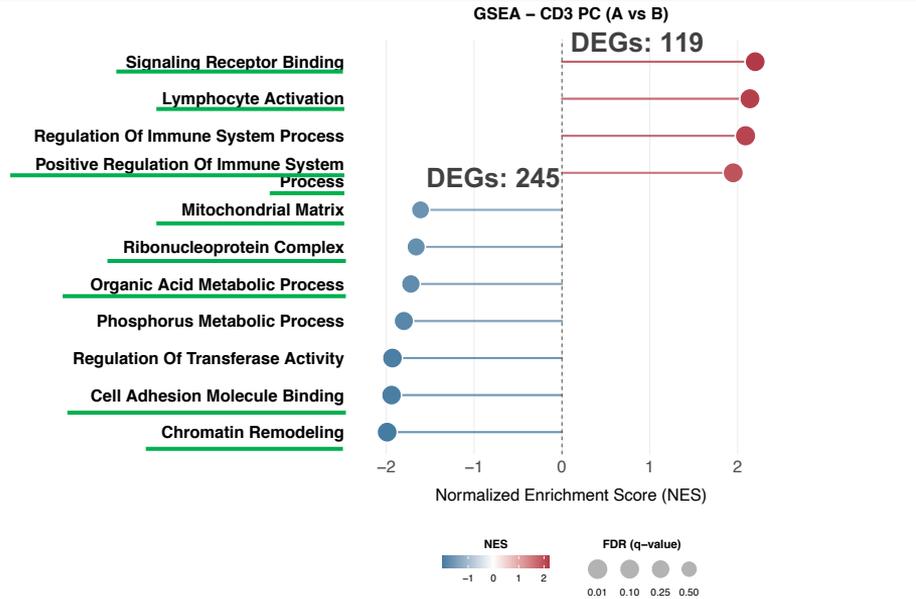- **Group C (R/R):** Motility and locomotion pattern, apoptosis

EXTRA PC – CD68+ macrophages undergo transcriptional reprogramming during CLL progression

GSEA – CD68 ExtraPC (A vs B)

DEGs: 226

- Adaptive Immune Response
- Immune Effector Process
- Blood Vessel Morphogenesis
- Signaling Receptor Binding
- Inflammatory Response

DEGs: 238

- Chromatin Remodeling
- Ribonucleoprotein Complex
- Chromatin

Normalized Enrichment Score (NES)

NES   FDR (q–value)
-2 -1 0 1 2   0.01 0.10 0.25 0.50

GSEA – CD68 ExtraPC (B vs C)

DEGs: 43

- Nitrogen Compound Transport
- Translation

DEGs: 410

- Supramolecular Complex
- Supramolecular Polymer

Normalized Enrichment Score (NES)

NES   FDR (q–value)
-1 0 1   0.01 0.10 0.25 0.50

- **Group A (Indolent):** ↑ Cellular activation, immune signaling, angiogenesis
- **Group B (Need of treatment):** Chromatin remodeling, transcriptional control, metabolic activity
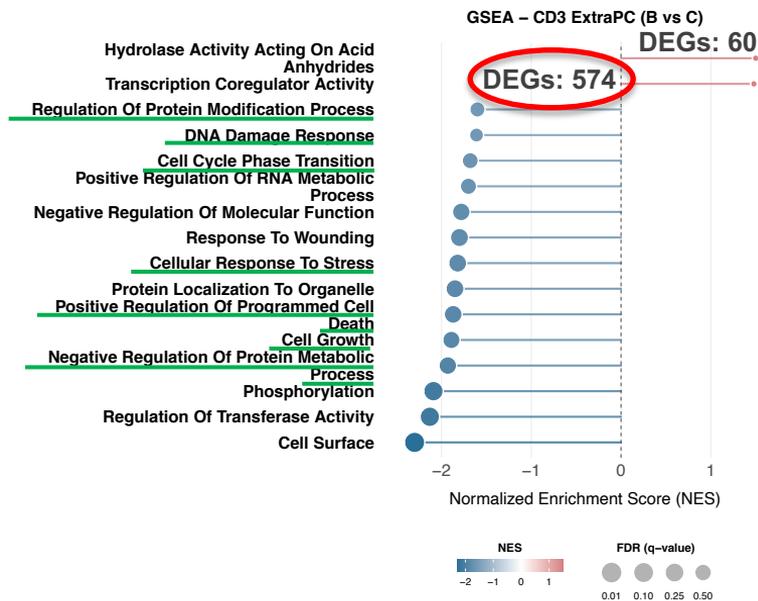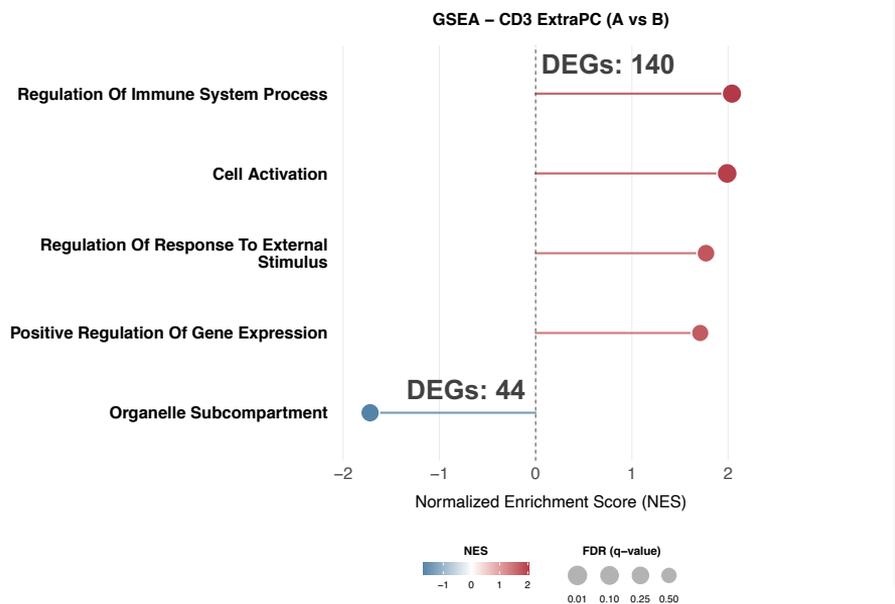- **Group C (R/R):** Structural functions

PC - Spatial profiling of **CD3+** T lymphocytes shows an evolution of the functional status during CLL progression

GSEA – CD3 PC (A vs B)

DEGs: 119

DEGs: 245

- Signaling Receptor Binding
- Lymphocyte Activation
- Regulation Of Immune System Process
- Positive Regulation Of Immune System Process
- Mitochondrial Matrix
- Ribonucleoprotein Complex
- Organic Acid Metabolic Process
- Phosphorus Metabolic Process
- Regulation Of Transferase Activity
- Cell Adhesion Molecule Binding
- Chromatin Remodeling

Normalized Enrichment Score (NES)

NES FDR (q–value)

GSEA – CD3 PC (B vs C)

DEGs: 112

DEGs: 161

- RNA Processing
- Protein Containing Complex Organization
- DNA Metabolic Process
- Protein Dimerization Activity
- Cell Motility
- Anatomical Structure Formation Involved In Morphogenesis
- Organelle Subcompartment
- Vesicle Membrane

Normalized Enrichment Score (NES)

NES FDR (q–value)

- **Group A (Indolent):** Immunological, activated profile [↑ immune competence]
- **Group B (Need of treatment):** Metabolic and cellular activation, mitochondrial activity, chromatin remodeling
- **Group C (R/R):** Vesicular trafficking, morphogenesis and motility

EXTRA PC - Transcriptional shift of **CD3+** T lymphocytes emerges primarily in progressed CLL

GSEA – CD3 ExtraPC (A vs B)

DEGs: 140

DEGs: 44

Normalized Enrichment Score (NES)

GSEA – CD3 ExtraPC (B vs C)

DEGs: 60

DEGs: 574

Normalized Enrichment Score (NES)

- **Group A (Indolent)/B (Need of treatment):** No significant changes
- **Group C (R/R):** Cell signaling, metabolism, stress response, growth, apoptosis, cell cycle, DNA repair, post-translational modifications.
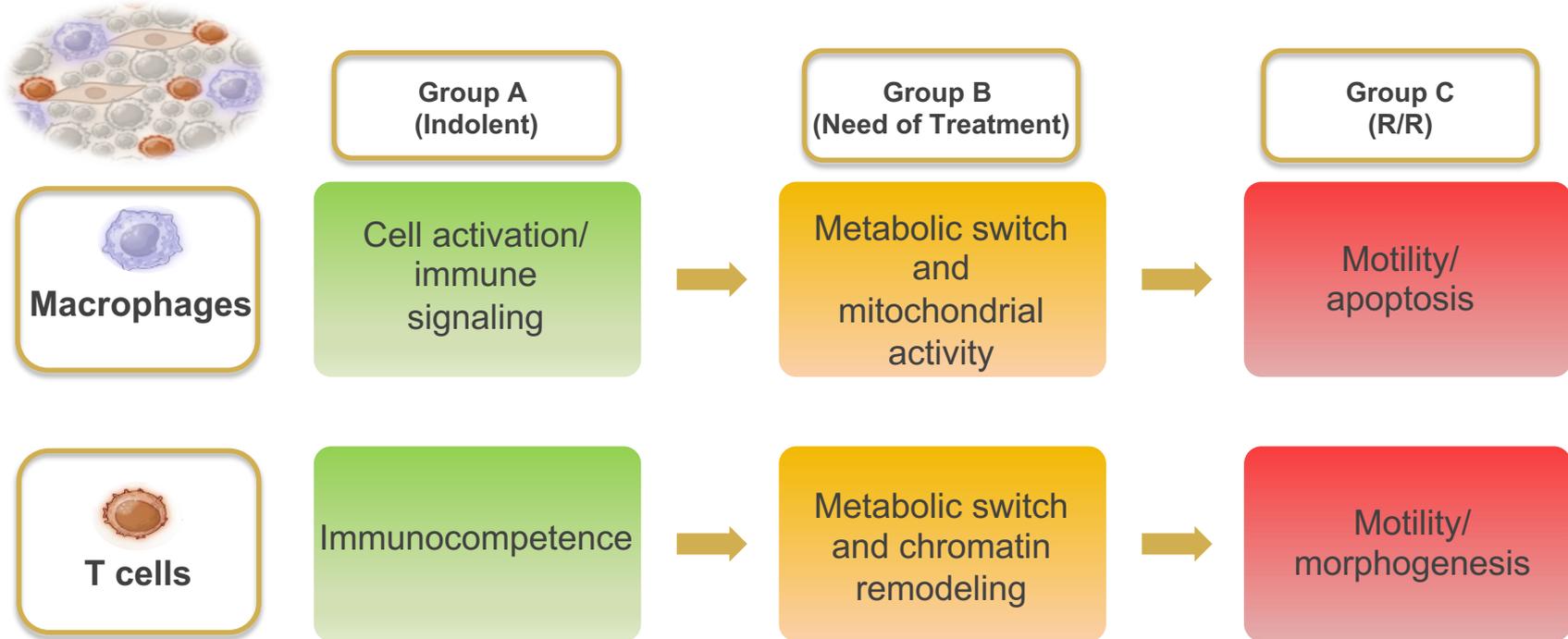
Work in progress…

Profile transcriptional programs of TME cells across disease states

Identify the spatial contribution of individual cell types to the state and progression of the disease

Characterize dynamic tumor microenvironment interactions

Identify a predictive molecular signature → validation

Cell state analysis

Interaction analysis

Signature selection and validation

Acknowledgement

Hematology Unit
Carla Minoia
Maria Carmela Vegliante
Antonella Bucci
Giulia Notarpietro
Susanna Anita Pappagallo
Paolo Mondelli
Sabino Ciavarella
Attilio Guarini
Rare Tumors and Melanoma Unit
Benedetta Apollonio
Pathology Unit
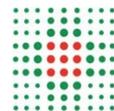Rossana Daprile
Anna Scattone

Pathology Unit
Maurilio Ponzoni
University Vita-Salute San Raffaele
Paolo Prospero Ghia
B-cell Neoplasia Unit Division of Experimental Oncology
Michela Frenquelli

Laboratory of Translational Research
Alessia Ciarrocchi
Benedetta Donati

Giancarlo Castellano

Piano Nazionale
di Ripresa e Resilienza
PNRR-MAD-2022-12376441

Thank you for your attention!