#1212. High-dimensional profiling of CLL and T cell interactions during early phase of disease using a 42-color full-spectrum cytometry panel

S. Mallorquí-Alcalá[1], I. Salvador[1], C. Palacio[1,2], M. Bastos-Boente[3], M. Alcoceba[3], C. de Ramón[3], A. Navarro[3], D. Medina-Gil[1], P. Fernández-Guzmán[1], M. Erguin[1], Á. Serna[1,2], P. Abrisqueta[1,2], F. Bosch[1,2], M. Crespo[1,2]

1 Experimental Hematology, Vall d'Hebron Institute of Oncology (VHIO), Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain. 2 Hematology Department, Vall d'Hebron Hospital Universitari, Vall d'Hebron Barcelona Hospital Campus, Barcelona, Spain. 3 University Hospital of Salamanca (HUS/IBSAL), CIBERONC and Cancer Research Institute of Salamanca-IBMCC (USAL-CSIC), Salamanca, Spain

BACKGROUND

CLL cells behave as regulatory B cells, altering the functionality and proportion of immune subsets, especially T cells[1]. T lymphocytes from CLL patients exhibit dysregulated expression of activation molecules and increased expression of exhaustion markers[2]. Previous evidence from our group suggests that immune dysfunction, particularly T cell exhaustion, is increased at disease progression[3]. Therefore, we hypothesized that progressive immune dysfunction during the first months after diagnosis can identify patients at higher risk of early progression. To assess that, we longitudinally analyzed patient samples at diagnosis and six months later to correlate immune dysfunction with progression risk.

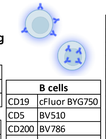
METHODS

Blood collection (n=34)

Cryopreservation

Full-spectrum cytometry

Surface + intracellular staining



Cleanup	
Viability	Zombie NIR
CD45	NovaFluor B690

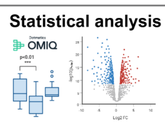
Monocytes and NKs	
CD11b	eFluor506
CD155	BUV615
CD14	BUV395
CD16	RB613
CD56	BUV737

B cells	
CD19	cFluor BYG750
CD5	BV510
CD200	BV786
CD38	PC5.5
CD49d	BV750
ZAP70	PE
Ki67	BV650
PD-L1	R718

T cells	
CD3	RB545
CD4	cFluor B532
CD8	SparkUV 387
CD45RA	Pacific Blue
CD95	APC-Cy7
CCR4	BUV805
CCR6	BUV563
CCR7	PE-CF594
CXCR3	APC-fire810
CXCR4	BV605
CXCR5	APC-R700
CD127	PC5
CD25	RB705

TCF7	AF647
CD270	BV711
CD48	BV421
HLA-DR	BV570
CD86	RY586
DNAM-1	BUV661
PD-1	RB744
TIGIT	PE-Fire 810
TIM-3	BB515
LAG-3	NovaFluor Y730
CD160	APC
CD244	PC7
BTLA	BV480
CTLA-4	RB780

Statistical analysis



Retrospective patient classification

Non-progressors (n=10)
Median follow-up: 99 mo

Progressors (n=11)
Time to progression: 79 mo

RESULTS

Longitudinal analysis reveals stable frequencies of main immune cell subsets except for monocytes

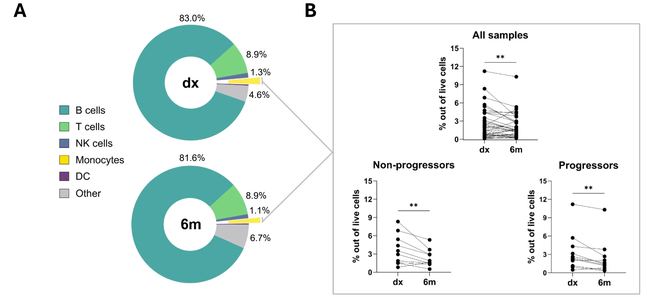


***Figure 1.* Overview of the immune system of CLL patients at diagnosis (dx) and after 6 months (6m).** (A) Pie charts showing the proportions of major immune cell populations among live cells at dx and 6m, with all remaining stable except for monocytes (n=34). (B) Change in monocyte abundance over time ($p < 0.01$).

PD-1, but not chemokine receptors or prognostic markers, shows dynamic expression on CLL cells over time

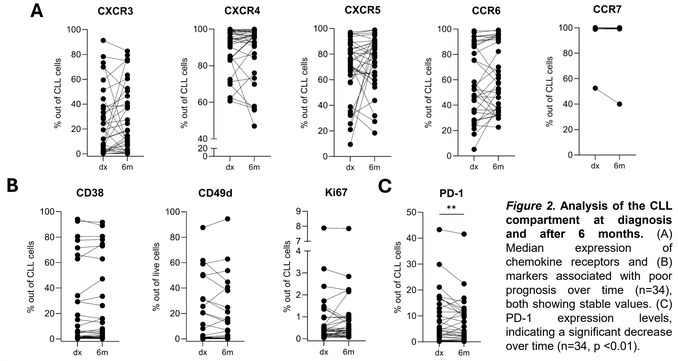
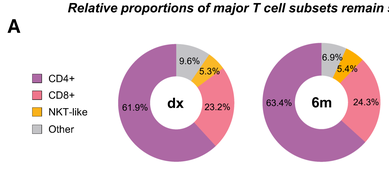


***Figure 2.* Analysis of the CLL compartment at diagnosis and after 6 months.** (A) Median expression of chemokine receptors and (B) markers associated with poor prognosis over time (n=34), both showing stable values. (C) PD-1 expression levels, indicating a significant decrease over time (n=34, $p < 0.01$).

Relative proportions of major T cell subsets remain stable over time, except for a decline in naïve CD4 and an increase in EM CD8⁺ in progressors



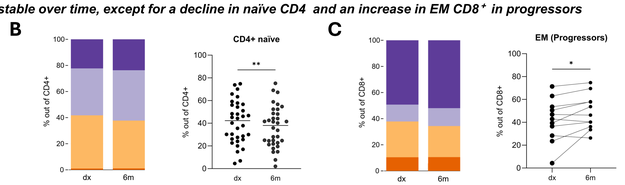
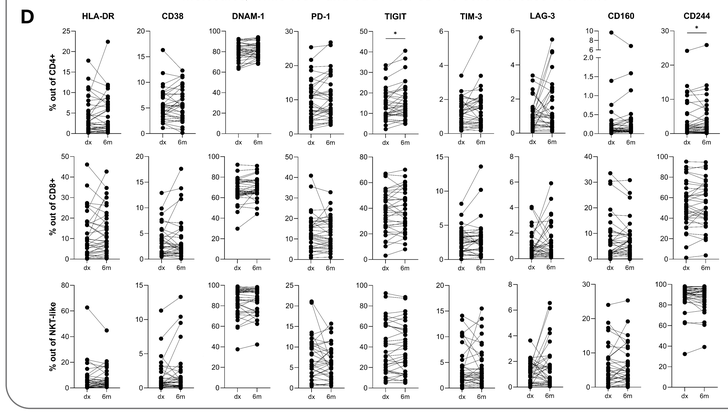
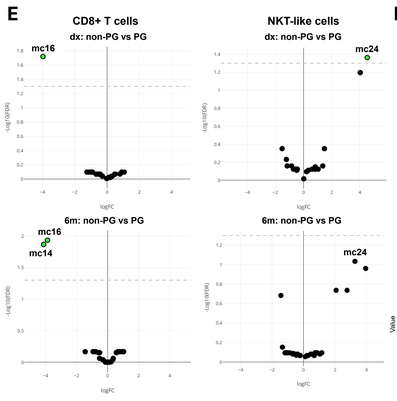


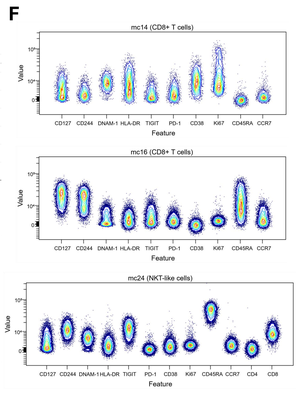
***Figure 3.* Longitudinal analysis of T cell populations at diagnosis and 6 months later.** (A) Pie charts showing stable proportions of T cell subsets across timepoints. (B) Relative proportions of CD4+ and (C) CD8+ T cell maturation subsets (n=34) are constant, except for naïve CD4+ T cells ($p<0.01$) and effector memory (EM) CD8+ T cells in progressors ($p<0.05$), which show significantly decrease and increase, respectively, over time. (D) Longitudinal expression of activation markers and immune checkpoint molecules remains constant, although TIGIT and CD244 increase on CD4+ T cells ($p<0.05$). (E) Differential abundance analysis of FlowSOM clusters in non-progressors (non-PG) versus progressors (PG) for CD8+ T cells and NKT-like cells (FDR<0.05). (F) Markers used for annotating significantly abundant clusters.

Major T cell subsets display stable expression of activation markers and immune checkpoint molecules, while TIGIT and CD244 on CD4⁺ T cells increase over time



Disease group comparisons reveal significant CD8⁺ and NKT-like cell clusters at both timepoints





CONCLUSIONS

Manual analysis suggest that disease progression in CLL is associated with changes in cell function rather than with alterations in the proportions of main immune subsets. Considering the phenotype of significant metaclusters obtained from the unsupervised analysis, those overrepresented in progressors reflect an exhausted profile, whereas in non-progressors, they exhibit activation features. However, ongoing analyses are required to elucidate potential immune changes occurring early after CLL diagnosis that may help predict disease progression.

REFERENCES

1. Mékinian A, Quinquenel A, et al. Immuno-regulatory malignant B cells contribute to Chronic Lymphocytic Leukemia progression. Cancer Gene Ther. 2023;30(6):1018-1028.
2. Riches JC, Davies JK, et al. T cells from CLL patients exhibit features of T-cell exhaustion but retain capacity for cytokine production. Blood. 2013 Feb 28;121(9):1612-21. Epub 2012 Dec 17.
3. Jiménez I, Tazón-Vega B, et al. Immunological and genetic kinetics from diagnosis to clinical progression in chronic lymphocytic leukemia. Biomark Res. 2021 May 20;9(1):37.

ACKNOWLEDGEMENTS

La Marató · 3CAT · XXI iwCLL International Workshop on CLL · ISC Instituto de Salud Carlos III

sofiamallorqui@vhio.net