XIX CONGRESSO NAZIONALE
SIES 2026

IL SINGLE-CELL SEQUENCING RIVELA PATHWAY PREDITTIVI DI DISSEMINAZIONE AL SISTEMA NERVOSO CENTRALE (SNC) NELLA LEUCEMIA LINFOBLASTICA ACUTA A CELLULE B

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Disclosures of L. V. Cappelli

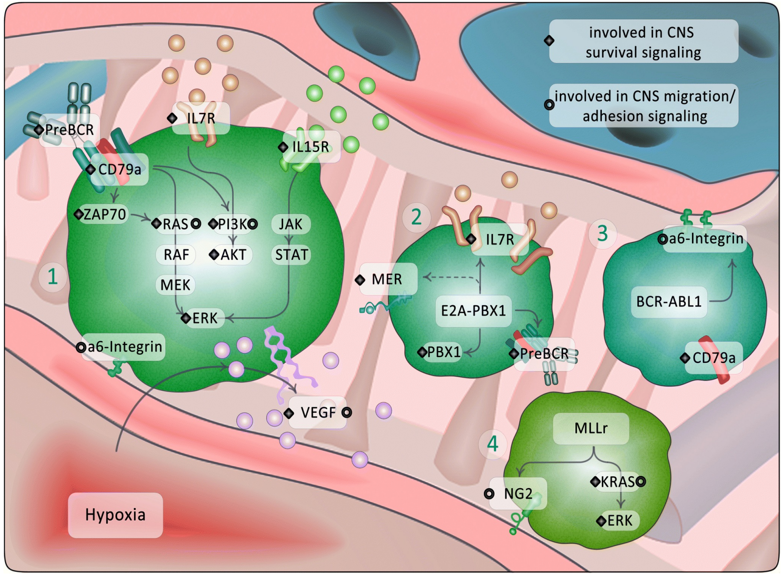
No conflicts to declare

CNS dissemination in acute lymphoblastic leukemia (ALL)

- CNS+: 5%–10% at diagnosis and 30%–40% at relapse

→ Role of IT/CHT prophylaxis

- Unfavorable prognosis, few effective drugs
- Blina e InO limited activity
- CD19 CAR-T show significant activity (ORR of 70-80% in active involvement)



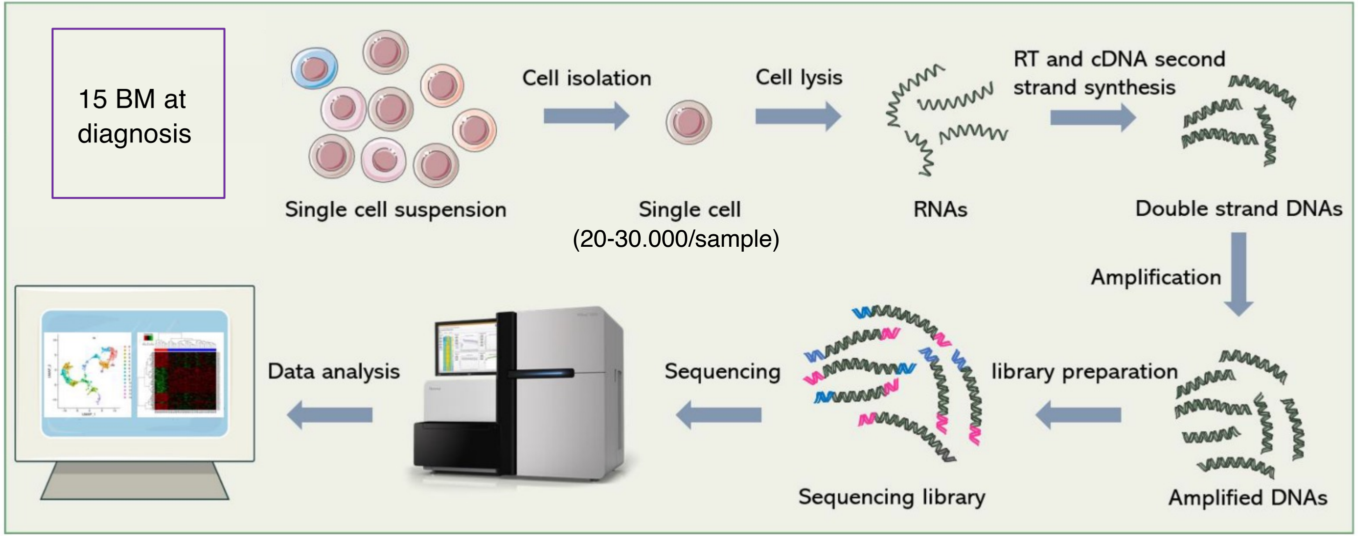
Lenk L et al, Canc & Metast Rev, 2020

Methods and Objectives

Objective: to characterize CNS dissemination using innovative *single-cell sequencing* technologies

Methods: Single-cell RNA sequencing (Singleron® Matrix NEO) on 15 diagnostic BM B-ALL samples

Bioinformatic analyses: DEGs («CNS gene set»), UMAP, *pseudotime* e *cell-type inference*



Study cohort

	n= 15
Male, (%)	10 (67%)
Age at diagnosis, median (range)	45 (18-60)
% Bone marrow Blast infiltration, median (range)	83 (62-96)
WBC at diagnosis, median (range)	24.8 (0.2-161)
Lineage by flow-cytometry	
Pro-B	1 (6.7%)
B-Common	13 (86.6%)
Pre-B	1(6.7%)
Molecular aberrations	
Ph-like	4 (30.7%)
t(4;11)	1 (6.7%)
Cytogenetics	
Complex Karyotype	3
Normal	2
Other	4
NA	6

Fusion transcripts	4
CRLF2::P2Y38	1
MEF2D::BCL9	1
IKZF1::DDC (+*CRLF2*-r FISH)	1
PAX5::ZCCHC7	1
IKZF1 status	
None	4
Loss	6
IKZF1^{plus}	3
NA	
Relapse status	
CNS_R	4 (30.7%)
BM/CNS_R	2 (13.4%)
BM_R	3 (20%)
No relapse	6 (40%)
Median follow-up	8 months (4-117)
Death/Alive	3/12

CNS status:	
No dissemination	6
BM relapse	3
CNS relapse	4
BM&CNS relapse	2

Generation of a «CNS gene set»

CNS+

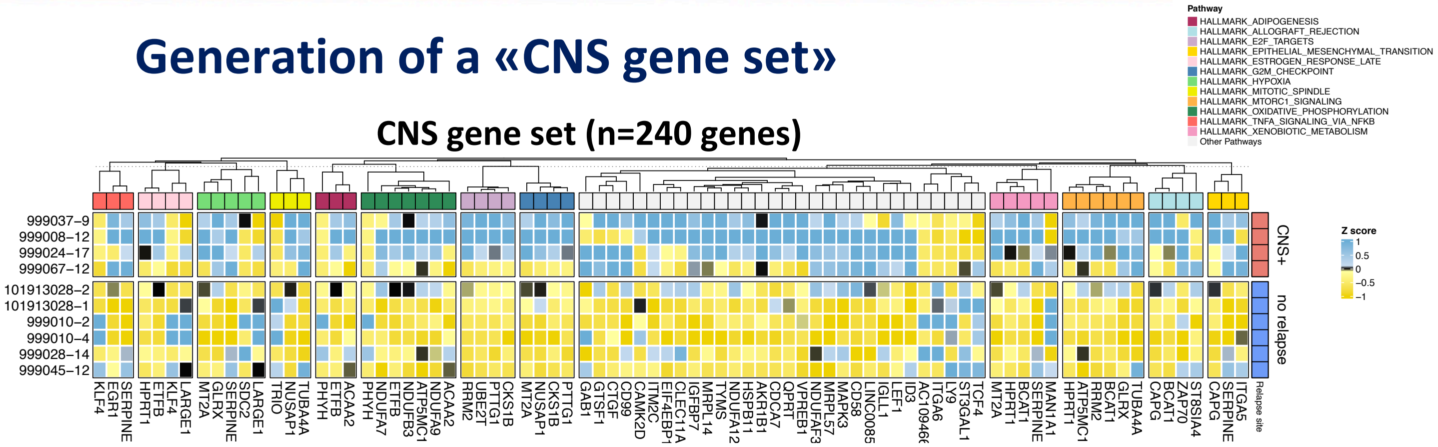
n=4 CNS+ only

vs

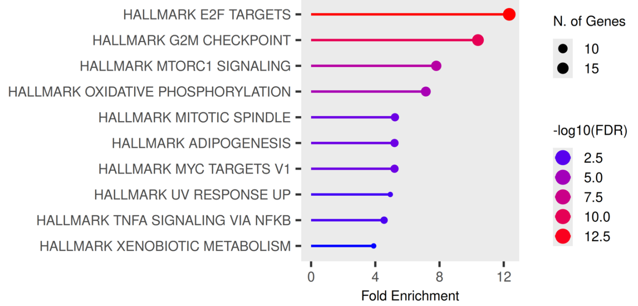
CNS-

n=6

CNS gene set (n=240 genes)



CNS signature enriched genes



Individual upregulated genes in CNS+

ZAP70[1]

CTGF[2]

VPREB1[3]

LEF1[4]

ID3[5]

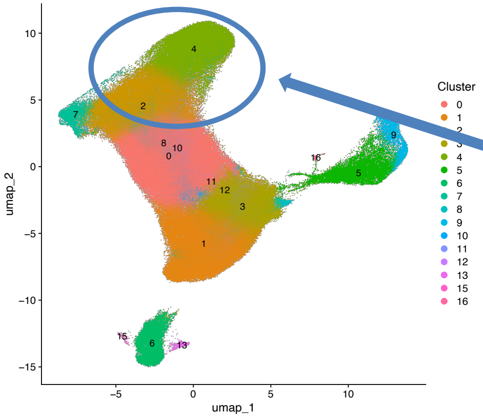
CD99[6]

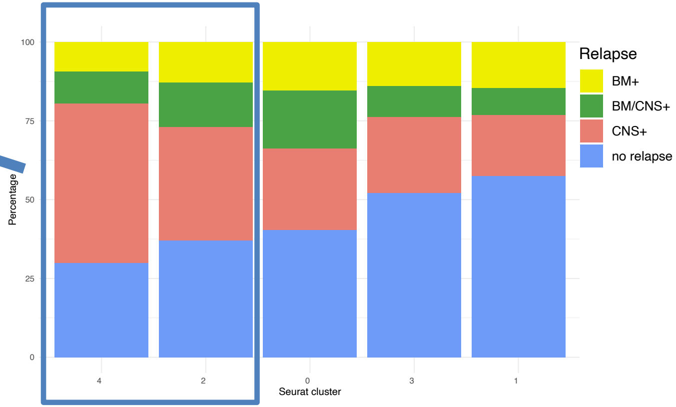
ITGA5[7]

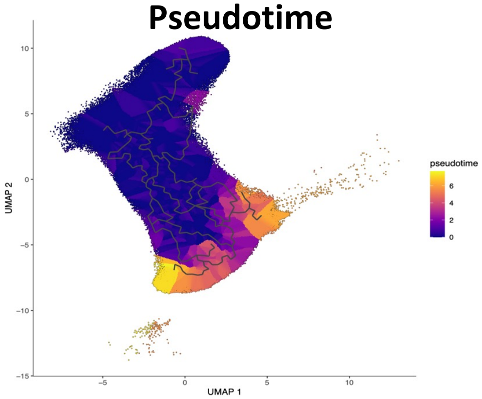
SERPINE1[8]

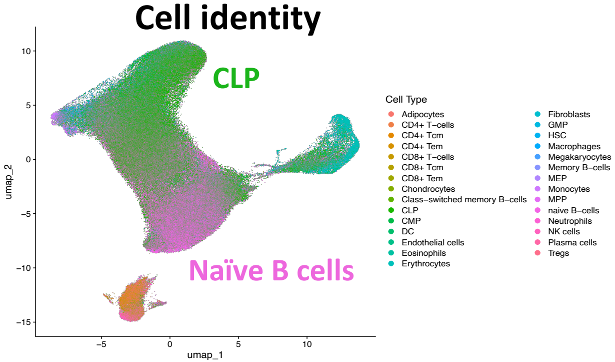
1. Alsadeq A, et al. *Haematologica* 2017
2. Sala-Torra. et al. *Blood* 2007
3. Gordon P. et al. *Blood Neoplasia* 2025
4. Kuhnl A, et al. *Blood* 2011
5. Poveda-Garavito, et al. *Front Immunol* 2024
6. Chen S, et al. Br J Haematol 2019
7. Wang P. et al. *OMCL* 2022
8. Wang B. et al. *Small* 2025

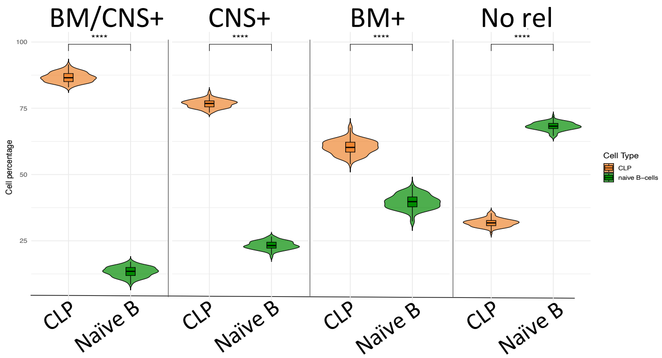
Immature blasts are enriched in CNS+ samples





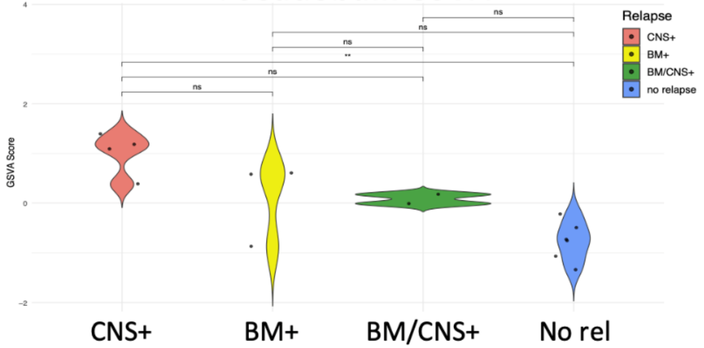

Pseudotime


Cell identity



CNS+/BM+: more immature (common lymphoid progenitor - “CLP”) vs no relapse (naïve B)

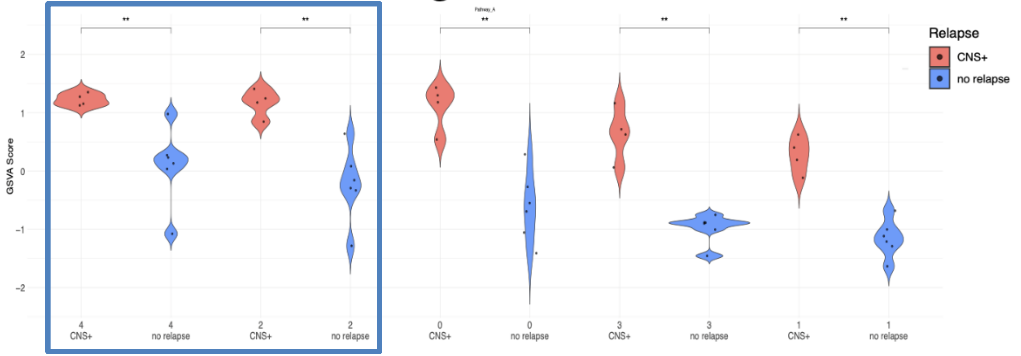
«CNS gene set» : evaluation at the single-cell level



Pseudobulk GSVA

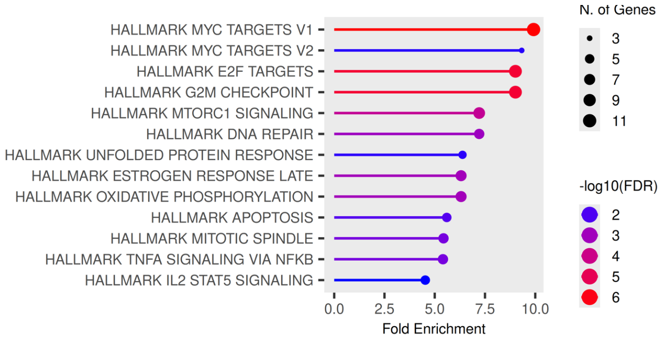
Clusters with more immature cells (#2, #4) upregulate the "CNS gene set" more than mature ones (#1, #3)

In the analysis restricted to the immature clusters, the "CNS gene set" remains differentially expressed in CNS+ vs CNS- samples

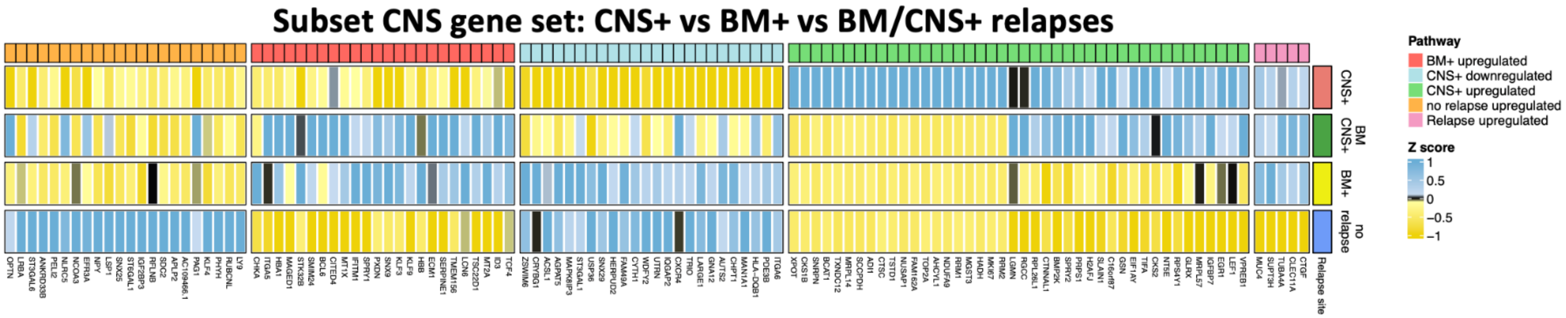


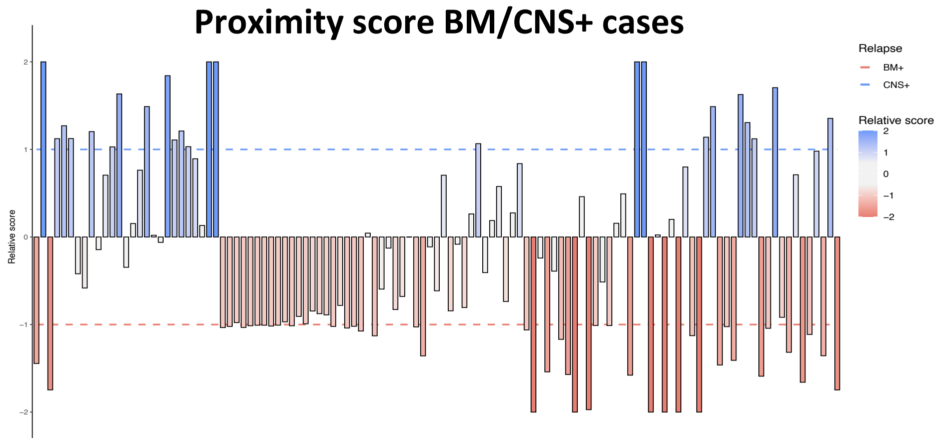
Single-cell GSVA

Immature clusters, CNS+ vs CNS-: upregulated genes



«CNS gene set»: comparison between CNS and BM relapse


Subset CNS gene set: CNS+ vs BM+ vs BM/CNS+ relapses


Proximity score BM/CNS+ cases

Pts with *CNS+ only relapse* show a signature partially shared with *BM+* e *BM/CNS+* relapses and partially exclusive

Pts with *BM/CNS+ relapse* show a hybrid profile between *CNS+* and *BM+* relapses, but closer to *BM+*

Restricted CNS gene set

Generation of a restricted set and *proof of concept* validation

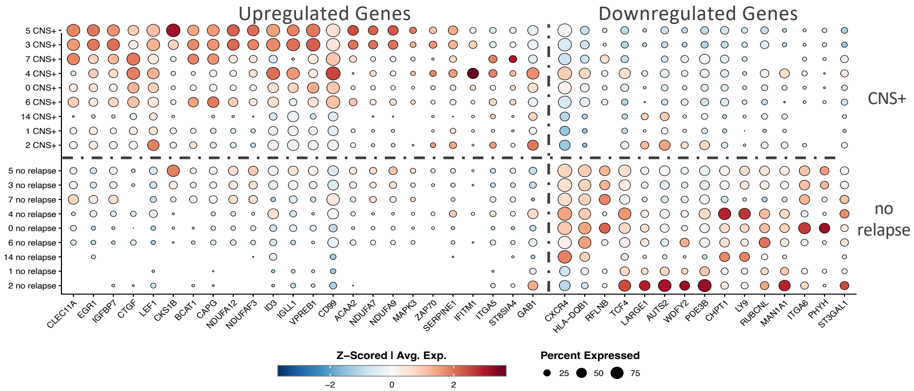
Genes, no. **240** >
- Weighted score
- Literature

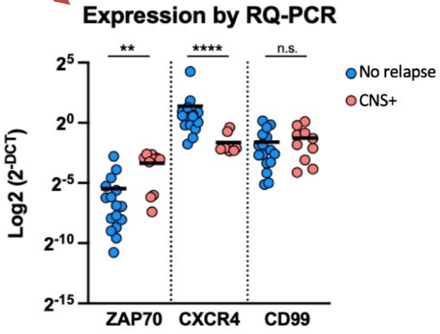
> **39** > **3**

ZAP70[1]
CXCR4[2]
CD99[3]

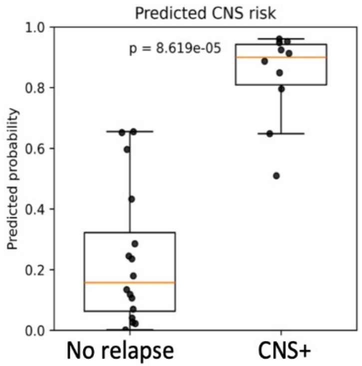
N=26 pts

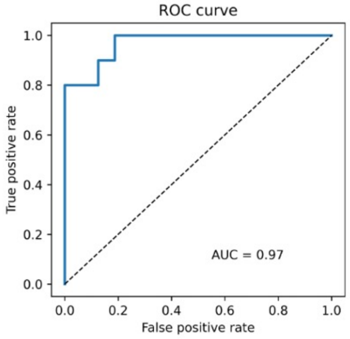
Restricted 39-genes set





Kaiser F et al, SIES 2026; *Poster p034*





Final objective ➔ ***a model predictive of CNS dissemination to be implemented in trials***

1) Chiaretti et al, *Blood*, 2006; 2) Ayala et al, *Leukemia*, 2009; 3) Chen et al, *Br J Haematol*, 2019

Conclusions

- **CNS gene set** stratifies CNS+ relapsing vs not relapsing pts on BM at diagnosis
- Single-cell: More immature clusters (CLP) are enriched in CNS+ patients
- These clusters upregulate the **CNS gene set**

↓

- **Patients who have dissemination to the CNS present, from the moment of diagnosis, cells in the BM with biological characteristics and favorable tropism for the CNS.**
- **To develop a CNS risk predictive tool to be tested in prospective clinical trials to guide therapeutic decisions**

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