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Title Page

Leukemia stem cells in acute myeloid leukemia-mimicking "space and time continuum"?

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"Time and space are not conditions of existence, time and space are models for thinking." Albert Einstein

It's been 100 years since Einstein's "space-time" theory of relativity was published. In physics, the space-time continuum is a mathematical model that merges the *three dimensions of space* and the *single dimension* of time into a *single four-dimensional continuum*. Space-time diagrams are useful for visualizing relativistic effects, such as how different observers perceive *"where" in space* and *"when" in time,* events occur.

We can probably draw an analogy with the dimension of "*where*" to capture the MRD (Measurable Residual Disease) and "*when*" (time point) to check it, and do different methods and observers (flow labs & clinicians) perceive this in the same way?

Beside molecular techniques and flow cytometry (MFC) using the LAIP/Dfn strategy[1], the concept of LSC (Leukemia Stem Cells) monitoring appears to be a new "dimension" in the longitudinal therapeutic follow-up of AML (Acute Myeloid Leukemia).

It's been 30 years since Dick and colleagues demonstrated the heterogeneity of leukaemia "bulk" , describing leukemic stem cells CD34+CD38- with stemness characteristics, with potential of generating leukaemia in immunodeficient mice, based on leukemogenesis hierarchical or stochastic models [2,3] .

In this issue of Haematologica, Ngai *et al* [4] describe the prognostic value on overall survival and incidence of relapse of different methods of LSC quantification across ELN2017 risk groups, using data from the HOVON-SAKK132 trial.

This well performed study provides data from a large cohort of patients allowing robust results highlighting the prognostic relevance and clinical impact of LSC flow-based assessment in AML at diagnosis and follow-up. The authors underlines as key points the methodological aspects in LSC measurement: 1) defining the number of target events to improve sensitivity 2) different threshold levels for LSC positivity assessment; 3) changing the denominator for primitive marker-positive cells (PM, CD34+ cells) instead of WBCs; 4) changing the CD38 negativity threshold for CD34+CD38- cells.

The most important question concerned the impact of these adjustments on prognostic value in the different ELN risk groups.

LSC are rare events and detection by MFC should obey to strict rules, implying to use "Poisson Law" to verify the statistical accuracy of the results. To have confidence in the data we need to be sure that the events being counted are "truly" events of interest and not "random" events falling into the gates of interest[5]

In Poisson statistics, the major point is the number of positive events, conditioning the total number of events to confirm that the results are real and not random. There is no arbitrary number of events that is the "right" number[6]: knowledge of the data generated by your controls (a set of reference healthy Bone Marrows) define the LOB (Limit of Blank) corresponding to random events generated during the test. Ex, 10 to 15 positive events may be sufficient, if this number is significantly higher than the LOB, thus allowing for correct interpretation of the data and avoiding "false positives" events.

The authors aim to evaluate firstly, the minimum number of acquired cells for accurate LSC measurement and secondly , the value of individual LSC markers in order to delineate the effect of adjustments to the LSC flow strategy CD34+CD38- on the prognostic relevance of LSC at diagnosis and in follow-up. They agree that a threshold of "one" event is challenging to determine positivity and could be very difficult to standardized to be applied in a clinical setting. Furthermore, the acquisition level of 1 million WBC45+ seems realistic to achieve robust LSC quantification in clinical routine, improving patient eligibility for LSC evaluation in most standardized platforms, to obtain a LOD of 0.001%, a LOQ of 0.01% and a LSC cluster with a minimum of 10 events. The variation in LSC results shows the importance of LSC cluster size for positivity interpretation, to avoid false random positivity on "1" single-event cells.

The prognostic value of low-level LSC may change depending on genetic risk and treatment sequence. This is an important point highlighted in this study. Anyway, LSC measurement is now well established having a crucial prognostic value to apply at diagnosis and for MRD monitoring in all ELN risk groups combined to conventional MRD LAIP/Dfn measurement.

Previous literature provide data about LSC markers used in AML flow panels based on differential expression between nHSC and LSC *(Fig1)*. The authors used the HOVON LSC assay, to measure LSC fraction at diagnosis and during trial defined follow-up time points.

By exploring separate LSC markers, they observed that there is no universal LSC marker that can capture all LSC. However, other markers are described to identify LSC, in particular with functional characteristics, involved in stemness, dormancy, proliferation and chemoresistance[7]. This could be achieved using last generation cytometers (Spectral Flow) and new strategy of analysis using software with high dimensional data algorithms. Recommendations and guidelines are ongoing in the ELN David Flow group[8].

More interesting, there was observed variability in LSC cells population dynamics between diagnosis during treatment and at relapse, frequently, the LSC fraction being enriched at relapse time. *(Fig2)*

Einstein once said, *"Time and space are not conditions of existence; time and space are models for thinking."* In the same way, we need a new way of thinking about the MRD, one that includes looking for rare leukemia-initiating cells that play a role in relapse as a new dimension in monitoring the MRD. The authors have demonstrated that capturing these rare events contributes to a more accurate classification of patients at high risk of relapse, regardless of their ELN risk group. However, it is necessary to carefully interpret the LSC data based on the cut-off of MRD LSC positivity and on the CD38 level thresholds.

According to plasticity capacity moving in one way from CD38+*dim* to CD38-*neg* fraction and inversely, as reported by J Dick *et al*[9], the authors highlighted another interesting point when applying different CD38 levels according to the different ELN risk groups to identify patients with MRD LSC positivity. This plasticity could be linked to genomic patterns of mutations also to microenvironment and immune escape.

It's been 20 years since Gerrit Schurhuis *et al,* one of the pioneers of the application of LSC CD34+CD38- in AML in clinical use[10] and particularly in MRD LSC follow-up (MRD scoring LAIP/Dfn and LSC). Higher percentage of chemotherapy resistant LSC cells could lead to the outgrowth of MRD LSC in relapse.

As reported in this elegant study by Ngai *et al,* to prepare LSC for clinical decision making more research is warranted, in particular for specific ELN risk groups or associated to new therapeutic landscape including low-intensity therapies (Venetoclax based regimen), and specific target molecules (FLT3, IDH, Menin…) and paying particular attention to LSC concept and potential clonal evolution[11] In order to better understand AML clonal resistance and target the "scapegoat" cells, monitoring LSC flow may offer a new *"dimension"* in the AML therapeutic landscape.

References :

1) Heuser M, Freeman SD, Ossenkoppele GJ, et al. 2021 update on MRD in acute myeloid leukemia: a consensus document from the European LeukemiaNet MRD Working Party. Blood. 2021;138(26):2753-2767.
2) Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med. 1997;3(7):730-737.
3) Dick JE. Stem cell concepts renew cancer research. Blood. 2008;112(13):4793-4807.
4) Ngai LL, Reuvekamp T, Hanekamp D, et al. Different features of acute myeloid leukemia stem cell quantification in intensively treated patients. Haematologica. XXX
5) Hedley BD, Keeney M, Technical issues: flow cytometry and rare event analysis. Int J Lab Hematol . 2013;35(3):344-350.
6) Mario Roederer, How many events is enough? Are you positive? Cytometry A. 2008;73(5):384-385.
7) Hinchly T, Bonnet D, Anjos-Afonso F. Methodologic considerations on how to identify human hematopoietic stem cells. Exp Hematol. 2025;144:104729.
8) Tettero J M, Freeman S, Buecklein V, et al. Technical Aspects of Flow Cytometry-based Measurable Residual Disease Quantification in Acute Myeloid Leukemia: Experience of the European LeukemiaNet MRD Working Party. Hemasphere. 2022;6(3):e703.
9) McKenzie JL, Gana OI, Doedensa M, Dick JE. Reversible cell surface expression of CD38 on CD34-positive human hematopoietic repopulating cells. Exp Hematol. 2007;35(9):1429-1436.
10) van Rhenen A, Feller N, le Kelder A, et al, High Stem Cell Frequency in Acute Myeloid Leukemia at Diagnosis Predicts High Minimal Residual Disease and Poor Survival, Clin Cancer Res. 2005;11(18):6520-6527.
11) Stelmach P, Trumpp A. Leukemic stem cells and therapy resistance in acute myeloid leukemia. Haematologica. 2023;108(2):353-366.

Figures

Figure 1. Stem cells markers

Figure 2. Immunophenotype fractions and LSC (Leukemia Stem Cells) dynamic between diagnosis and relapse: genotype & phenotype heterogeneity

2A) Relapse Progenitors committed (AML « LSClow »): WT1+, NPM1-, FLT3ITD+, EVI1- , 46XX (idem diagnosis and relapse); (Phenotype identical relapse) ;

2B) Relapse Primitive cells (LSC) with clonal selection (AML « LSClow »): Diagnosis : WT1+, NPM1+, FLT3ITD-(VAF<1%) , DNMT3A+, EVI1- , 46XX; Relapse: WT1+, NPM1+, FLT3ITD+ (VAF 32%) , DNMT3A+, EVI1- , 46XX ; (Clonal selection relapse);

2C) Relapse Primitive cells (LSC) identical clone as diagnosis (AML « LSChigh ») : 42-44,XY,t(2;3)(p21;p21),-4,-5,add(6)(p23),der(16)t(?3;16)(q23;q22),-17,-18,-19,add(19)(p13),-21,+2-4mars[cp5] ;42-44,XY,t(2;3),del(5)(q13q32),-16,-17,-18,-19,-20,-21,+2-4mars[cp4] ; TP53+,VAF 34,5% ; (Clonal enrichment relapse) ;

Data adapted from: Plesa A, Roumier C; Gutrin J, et al. Measurable residual disease including AML leukemia stem cell flow evaluation of CPX-351 therapy by multi-parameter flow cytometry Leukemia Research 111 (2021) 106673

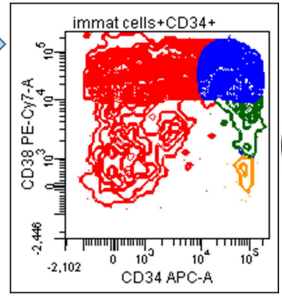
Figure 1

Identification
nHSC/hBM

Characterisation
LSC AML/Diag

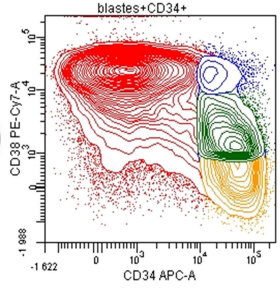
Monitoring
LSC AML/ MRD

LSC Flow Capturing: CD34+CD38- Space



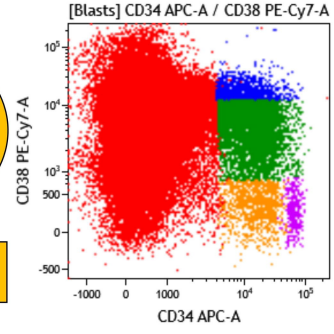
1963
Till & Mc Culloch

CD34+ CD38-



1997
John Dick & D Bonnet

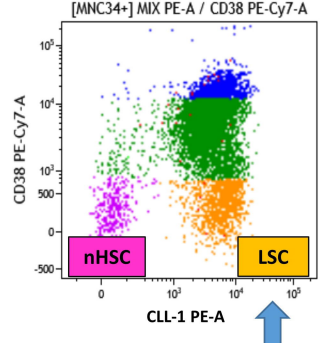
CD34+ CD38-



LSC Flow Quantification markers : dichotomy NEG vs POS

CD13+ lo CD33lo , CD117+
CD7-CD19-CD56-CD2-CD22-
CLL-1-, TIM3-, CD97-,CD123-/lo, CD45RA-

CD13+/-CD33++, CD117+/lo
CD7+CD19+CD56+CD2+CD22+
CLL-1+, TIM3+, CD97+,CD123++, CD45RA+



LSC Flow Fonctional markers

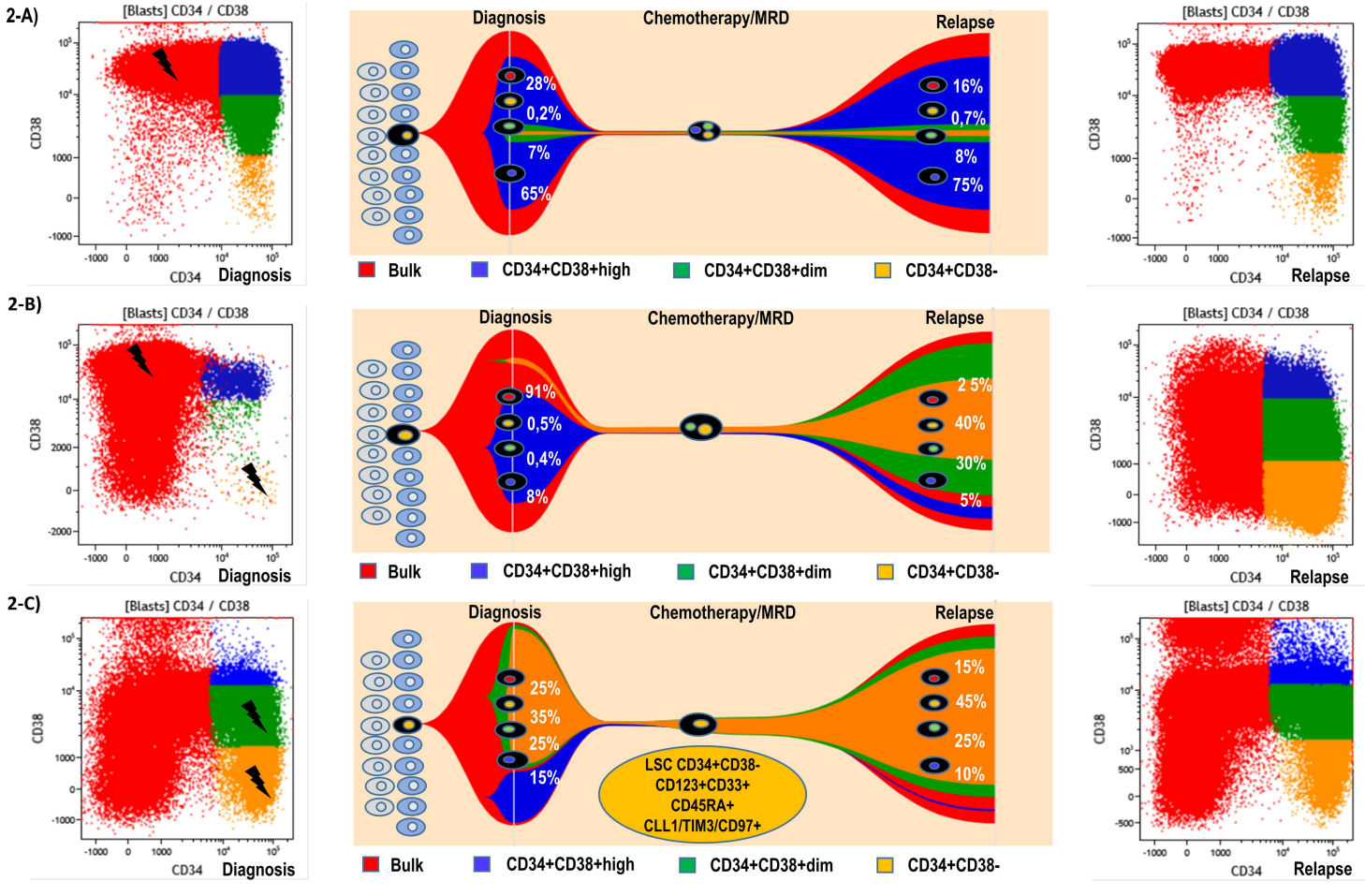
« Stemness »
Side Population (SP)
Hoechst 33342 , ALDH
Quiescence G0/G1
Xenograft, PDX

GPR56+	EPCR+
CD49f+	CD93+lo
CD81+lo	CD200-/+
CD44+	VLA4+
CXCR4+	CD47+
CD96+/-	CD25+/-

GPR56++	EPCR++
CD49f++	CD93++
CD81++	CD200++
CD44++	VLA4++
CXCR4++	CD47++
CD96++	CD25++

Targeting « scapegoat » cells

Figure 2


2-A)
[Blasts] CD34 / CD38
CD38
CD34
Diagnosis
Diagnosis
Chemotherapy/MRD
Relapse
28%
0,2%
7%
65%
16%
0,7%
8%
75%
Bulk
CD34+CD38+high
CD34+CD38+dim
CD34+CD38-
[Blasts] CD34 / CD38
CD38
CD34
Relapse
2-B)
[Blasts] CD34 / CD38
CD38
CD34
Diagnosis
Diagnosis
Chemotherapy/MRD
Relapse
91%
0,5%
0,4%
8%
2 5%
40%
30%
5%
Bulk
CD34+CD38+high
CD34+CD38+dim
CD34+CD38-
[Blasts] CD34 / CD38
CD38
CD34
Relapse
2-C)
[Blasts] CD34 / CD38
CD38
CD34
Diagnosis
Diagnosis
Chemotherapy/MRD
Relapse
25%
35%
25%
15%
LSC CD34+CD38-
CD123+CD33+
CD45RA+
CLL1/TIM3/CD97+
15%
45%
25%
10%
Bulk
CD34+CD38+high
CD34+CD38+dim
CD34+CD38-
[Blasts] CD34 / CD38
CD38
CD34
Relapse