Leukemia stem cells in acute myeloid leukemia - mimicking the “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 has 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 measurable residual disease (MRD) and “when” (timepoint) to check it, asking whether different methods and observers (flow laboratories and clinicians) perceive these in the same way. Beside molecular techniques and flow cytometry using the leukemia-associated immunophenotype/different from normal (LAIP/Dfn) strategy,[1] the concept of monitoring leukemia stem cells (LSC) appears to be a new “dimension” in the longitudinal therapeutic follow-up of acute myeloid leukemia (AML).

It has been 30 years since Dick and colleagues demonstrated the heterogeneity of leukemia “bulk”, describing $CD34^+CD38^-$ LSC with stemness characteristics, with the potential to generate leukemia in immunodeficient mice, based on hierarchical or stochastic models of leukemogenesis.[2,3] In this issue of *Haematologica*, Ngai *et al*.[4] describe the prognostic value, with regard to overall survival and incidence of relapse, of different methods of LSC quantification across European LeukemiaNet (ELN) 2017 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. As key points, the authors underline the methodological aspects of LSC measurement: (i) defining the number of target events to improve sensitivity; (ii) different threshold levels for assessing LSC positivity; (iii) changing the denominator to primitive marker-positive cells ($CD34^+$ cells) instead of white blood cells; and (iv) 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 and detection by flow cytometry should obey strict rules, implying the use of Poisson’s Law to verify the statistical accuracy of the results. To have confidence in data we need to be sure that the events being counted are true 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] the data generated by the controls (a set of reference healthy bone marrow samples) define the ‘limit of blank’, corresponding to random events generated during the test. For example, 10 to 15 positive events may be sufficient, if this number is significantly higher than the limit of blank, thus allowing for correct interpretation of the data and avoiding false-positive events.

The authors aimed firstly to evaluate 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 $CD34^+CD38^-$ LSC flow strategy 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 standardize in order for application in a clinical setting. Furthermore, the acquisition level of one million $CD45^+$ white blood cells seems realistic to achieve robust LSC quantification in clinical routine, improving patients’ eligibility for LSC evaluation in most standardized platforms, to obtain a limit of detection of 0.001%, a limit

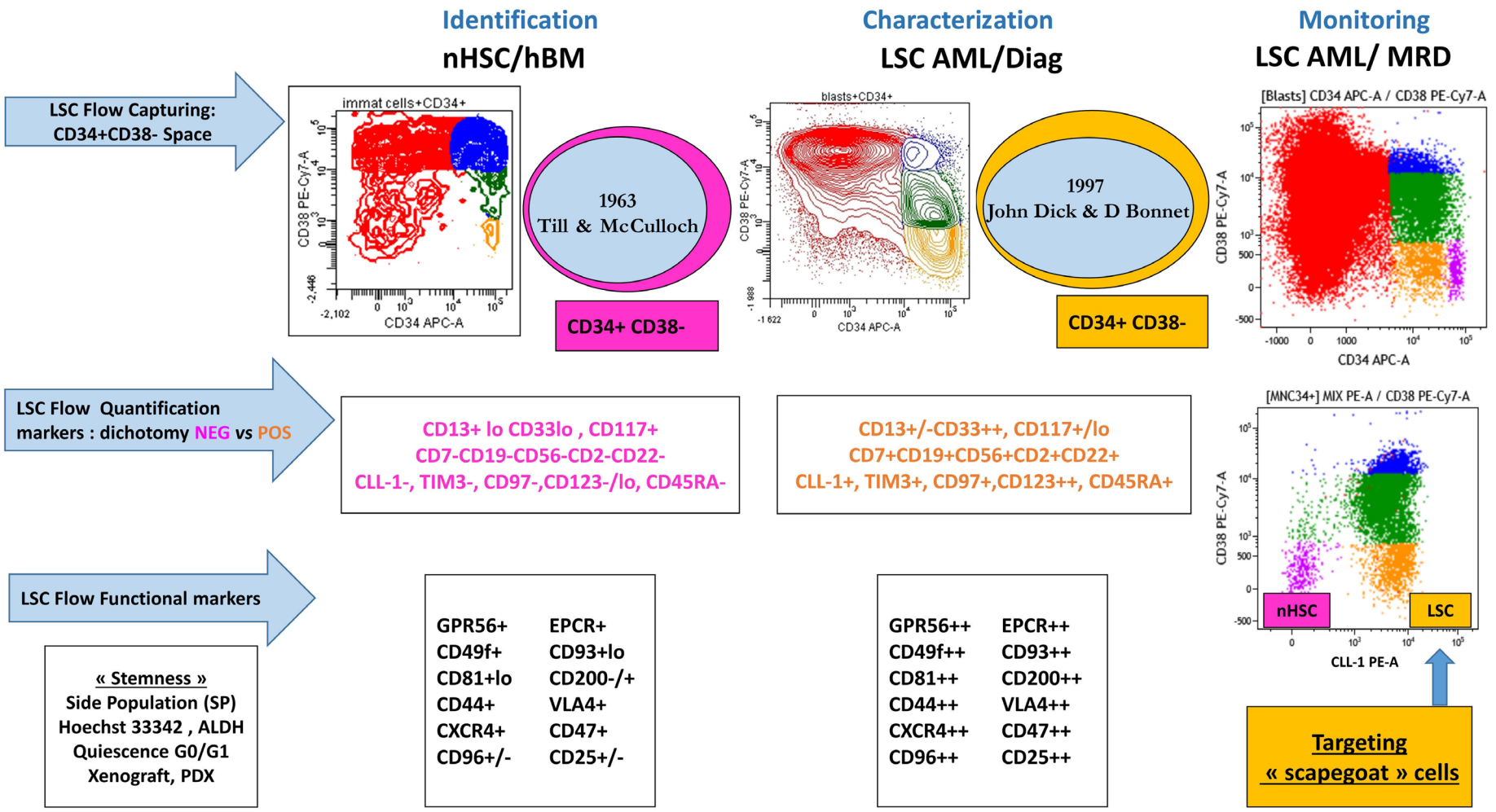

Figure 1. Stem cells markers. LSC: leukemia stem cell; immat: immature; PE-Cy7: phycoerythrin-cyanine 7; APC: allophycocyanin; nHSC: normal hematopoietic stem cells; hBM: human bone marrow; AML: acute myeloid leukemia; Diag: diagnosis; MRD: measurable residual disease; NEG: negative; POS: positive; MNC: mononuclear cells; ALDH: aldehyde dehydrogenase; PDX: patient-derived xenograft.

of quantification of 0.01% and a LSC cluster with a minimum of ten events. The variation in LSC results shows the importance of LSC cluster size for interpreting positivity, to avoid false random positivity on one single-event cell. The prognostic value of low-level LSC may change depending on genetic risk and treatment sequence. This is an important point highlighted in the study by Ngai *et al*.[4] Anyway, LSC measurement is now well established, having crucial prognostic value at diagnosis and for MRD monitoring in all ELN risk groups combined with conventional LAIP/Dfn MRD measurements.

Previous literature provides data about LSC markers used in AML flow panels based on differential expression between normal hematopoietic stem cells and LSC (Figure 1). The authors used the HOVON LSC assay to measure the LSC fraction at diagnosis and at defined follow-up timepoints during the trial.

By exploring separate LSC markers, they observed that there is no universal LSC marker that can capture all LSC. However, other markers have been described to identify LSC, in particular via functional characteristics, involved in stemness, dormancy, proliferation and chemoresistance.[7] This could be achieved using latest-generation cytometers (spectral flow) and new analytic strategies using software with high-dimensional data algorithms. Recommendations and guidelines are being prepared by the ELN David Flow group.[8]

More interesting, there was observed variability in LSC population dynamics between the time of diagnosis, during treatment and at relapse, with the LSC fraction frequently being enriched at the time of relapse (Figure 2).

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 MRD, one that includes looking for rare leukemia-initiating cells that play a role in relapse as a new dimension in monitoring MRD. Ngai *et al*.[4] 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, the LSC data must be interpreted carefully based on the cutoff of MRD LSC positivity and on the CD38 thresholds.

According to a capacity for plasticity, moving in one direction from a $CD38^{+dim}$ to a $CD38^{-}$ fraction and inversely, as reported by 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 as well as to the microenvironment and immune escape.

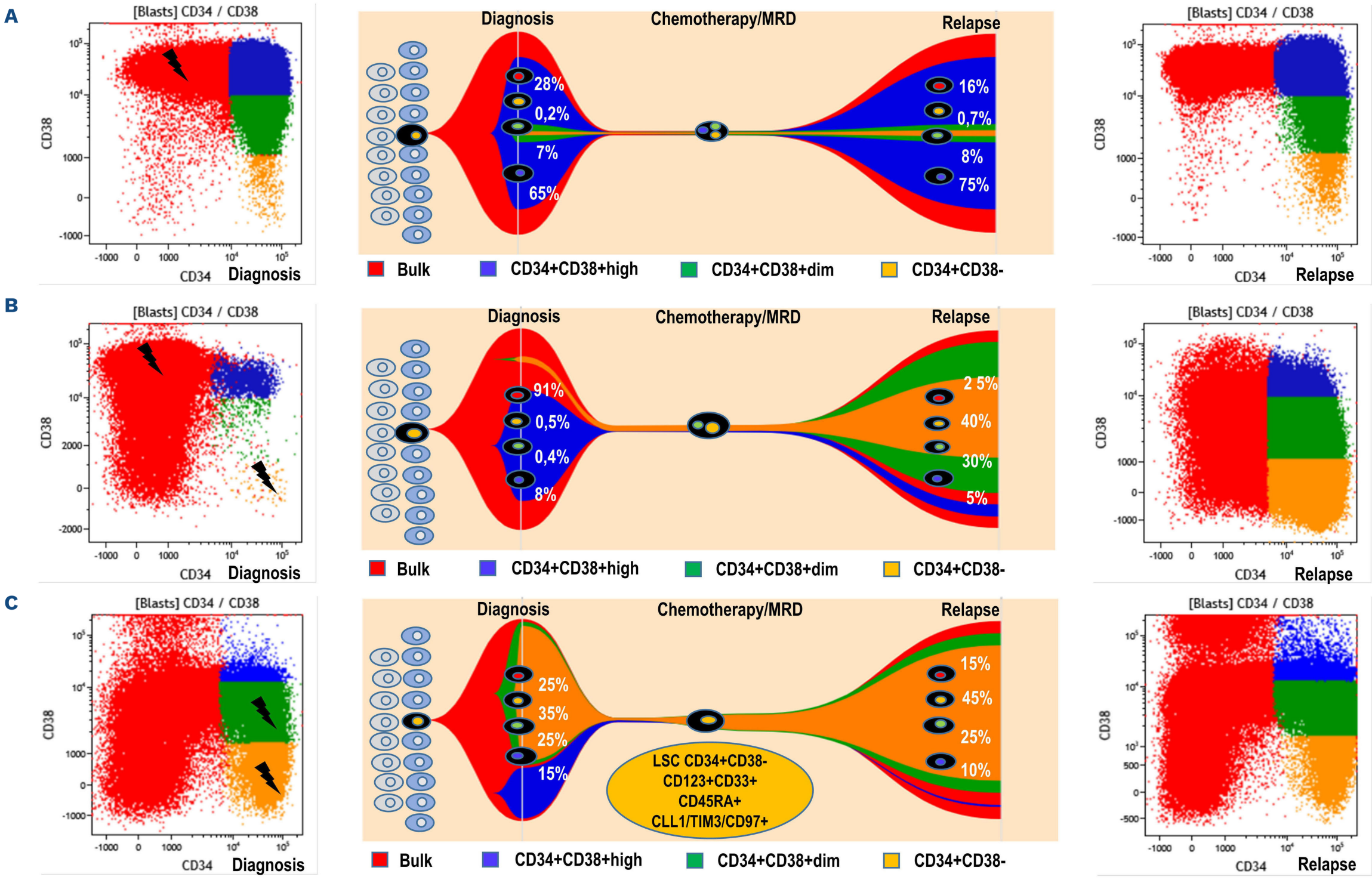

Figure 2. Dynamics of immunophenotype fractions and leukemia stem cells between diagnosis and relapse: genotype and phenotype heterogeneity. (A) Relapse – committed progenitors (AML «LSC^{low}»). At both diagnosis and relapse: WT1^{+}, NPM1^{-}, FLT3-ITD^{+}, EVI1^{-}, 46XX (phenotype identical relapse). (B) Relapse – primitive cells (LSC) with clonal selection (AML «LSC^{low}»). At diagnosis: WT1^{+}, NPM1^{+}, FLT3-ITD^{-} (VAF<1%), DNMT3A^{+}, EVI1^{-}, 46XX. At relapse: WT1^{+}, NPM1^{+}, FLT3-ITD^{+} (VAF=32%), DNMT3A^{+}, EVI1^{-}, 46XX (clonal selection relapse). (C) Relapse – primitive cells (LSC) with identical clone as diagnosis (AML «LSC^{high}»). 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). AML: acute myeloid leukemia; LSC: leukemia stem cells; VAF: variant allele frequency; MRD: measurable residual disease. Data adapted from: Plesa A, *et al*. Measurable residual disease including AML leukemia stem cell flow evaluation of CPX-351 therapy by multi-parameter flow cytometry. Leuk Res. 2021;111:106673.

Twenty years ago, Gerrit Schurhuis *et al*. pioneered the clinical use of $CD34^{+}CD38^{-}$ LSC assessment in AML[10] and particularly in MRD LSC follow-up (LAIP/Dfn MRD scoring and LSC). A higher percentage of chemotherapy-resistant LSC cells could lead to the outgrowth of MRD LSC in relapse.

As reported in the elegant study by Ngai *et al*.,[4] more research is warranted to prepare LSC monitoring for clinical decision-making, in particular for specific ELN risk groups or in the context of new therapeutic landscapes including low-intensity therapies (venetoclax-based regimens), and specific target molecules (FLT3, IDH, menin…) and paying particular attention to the concept of LSC 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.

Disclosures

No conflicts of interest to disclose.

Contributions

AP and CR contributed equally and co-wrote this Editorial.

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