UNIVERSITÀ DI SIENA 1240

Dipartimento di Scienze Mediche, Chirurgiche e Neuroscienze

Dottorato in Medicina Traslazionale e di Precisione

38° Ciclo

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IDENTIFICATION OF LEUKEMIC STEM CELLS IN AML PATIENTS BY USING NEXT-GENERATION FLOW: A PERSONALIZED BIOMARKER OF PROGNOSIS AND PROGRESSION

Settore scientifico disciplinare: MEDICINA E CHIRURGIA

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2024/2025

ABSTRACT

Acute Myeloid Leukemia (AML) is a biologically heterogeneous disease characterized by high rates of relapse: about 30% of AML patients achieving Minimal Residual Disease (MRD) negativity undergo a relapse, underlining the limitations of current MRD detection approaches and suggesting the persistence of leukemic cell populations that escape conventional monitoring, causing relapse in AML patients.

Leukemic Stem Cells (LSCs) represent a rare subpopulation within the $CD34^{+}CD38^{-}$ compartment and share several features with normal hematopoietic stem cells (HSCs). However, LSCs aberrantly express lineage and surface markers associated with leukemia, allowing their identification by Next Generation Flow (NGF).

This project aims to identify and characterize LSCs at baseline and to monitor them after induction therapy and after relapse, if any. Furthermore, this study tries to find a personalized LSCs MRD using NGF in order to investigate its prognostic value.

AML LSCs have been evaluated in bone marrow samples in AML patients using a combination of cytometry antigens (CD45, CD34, CD38, CD90, CD45RA, CD123, CD366 and CD371).

58 AML patients have been analyzed: 40/58 (69%) AML patients have been studied from diagnosis, confirming that, beside the bulk of leukemic clone, LSCs and residual HSCs can be identified and characterized using NGF. 29/40 (72,5%) AML patients were analyzed post induction therapy, monitoring AML-LSCs presence alongside treatment by using their specific LSCs-MRD panel: 20/29 (69%) still presented LSCs, instead 9/29 (31%) didn't show LSCs after the first course. Furthermore, we explored the role of LSCs persistence by comparing "LSCs MRD" to the residual AML clone using standard flow MRD and the gold standard molecular MRD markers in small specific clusters of patients. Finally, we explored also HSCs role after induction therapy, demonstrating that HSCs reappear only in some AML patients, suggesting a possible role as prognostic marker.

This is an exploratory pilot study confirming the possibility to detect AML LSCs using NGF, suggesting their role as possible biological MRD marker to monitor AML patients.

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ABBREVIATIONS

AML: Acute Myeloid Leukemia

BM: bone marrow

CLL-1: C-type lectin-like molecule-1

CR: complete response

ELN: European Leukemia Net

FLT3: FMS like tyrosine kinase

HSCs: hematopoietic stem cells

LDH: lactate dehydrogenase

LSCs: leukemic stem cells

MFC: multiparametric flow cytometry

MRD: minimal residual disease

NGF: next-generation flow cytometry

NGS: Next Generation Sequencing

NPM1: nucleophosmin 1

PD: progressive disease

PR: partial response

qPCR: quantitative PCR

SD: stable disease

Tim-3: T-cell immunoglobulin and mucin domain-containing protein 3

WBC: white blood cells

WT1: Wilms' Tumor

INTRODUCTION

Acute Myeloid Leukemia (AML) is a biologically heterogeneous disease characterized by high rates of relapse despite intensive induction chemotherapy. Although reaching a complete morphological remission represents the main therapeutic goal, disease relapse is the primary cause of failure [1]. In this context, minimal residual disease (MRD) assessment represents one of the powerful prognostic tools in AML [2]. Nevertheless, about 30% of AML patients achieving MRD negativity undergo a relapse, underlining the limitations of current MRD detection approaches and suggesting the persistence of leukemic cell populations that escape conventional monitoring [3,4]. Standard methods such as immunophenotype with multiparametric flow cytometry (MFC) and quantitative PCR (qPCR), are the main routine strategies for MRD monitoring in AML patients, in accordance with the international standard recommendations (European LeukemiaNet (ELN) 2022 guidelines) [2].

MFC approach allows MRD assessment by using a panel of fluorochrome labelled antibodies to distinguish leukemic blasts from normal myeloid precursors and to identify antigen express aberrantly on their surface including expression of antigens normally present on healthy myeloid cells. However, there are important limitations of MFC, such as the absence of aberrant immunophenotypic antigens to monitor over time or the high heterogeneity. Furthermore, standardized antibodies capable of assessing more sensitive MRD including detection of rare leukemic cells, are not yet available [1,5].

The qPCR technique is usually used for MRD monitoring with a detection sensitivity $\leq 10^{-5}$; however, the clonal heterogeneity in AML patients reduces its applicability for the MRD monitoring. Although the identification of additional mutations for developing patient-specific qPCR panels increased over the years, this does not guarantee the possibility of assessing MRD in all cases of AML [6].

Recent studies provide evidence indicating that relapse may be due to the presence of leukemic stem cells (LSCs), a small subpopulation of self-

renewing cells, leukemia-initiating cells, and cells resistant to cytotoxic therapy. It is thought that LSCs are able to escape chemotherapy by the quiescent nature of this cell population, a greater ability to repair DNA, altered metabolic activity, and interaction with bone marrow (BM) microenvironment. As a result, eradication of the bulk leukemic population does not necessarily eliminate the compartment responsible for leukemic propagation, providing a biological explanation for relapse despite MRD negativity [7-10].

LSCs represent a rare subpopulation within the $CD34^{+}CD38^{-}$ compartment and share several features with normal hematopoietic stem cells (HSCs), including self-renewal and lack of differentiation. However, unlike HSCs, LSCs aberrantly express lineage markers and surface markers associated with leukemia, allowing their identification by MFC. Despite this, LSCs remain highly heterogeneous between patients and disease subtypes, and a universally applicable immunophenotypic signature has not yet been defined, representing a major trouble to the standardization of their detection [11]. The LSCs quantification during treatment follow-up is technically challenging due to their extremely low frequency in BM samples. Previously proposed cut-offs for LSCs positivity are very stringent and require meticulous gating strategies to avoid non-specific events, especially when large numbers of cells are acquired. Moreover, variability in analytical approaches between studies, including differences in cut-off definitions or denominators used for LSCs quantification (total white blood cells versus $CD34^{+}$ cells), has hindered harmonization and limited the clinical translation of LSCs monitoring [12]. Published clinical applications of NGF include clinical trials employing high-dimensional patient-specific characterization of rare leukemic populations for comprehensive longitudinal immune monitoring of LSCs in parallel with standard MRD studies.

Several surface antigens including both C-type lectin-like molecule-1 (CLL1) and T-cell immunoglobulin and mucin domain-containing protein 3 (TIM-3) have been identified on LSCs. CLL-1 is selectively expressed on LSCs and mature myeloid cells but absent on normal HSCs, supporting its role as a prognostic biomarker and therapeutic target. Tim-3, an inhibitory immune

checkpoint receptor, is preferentially expressed on LSCs and has been shown to induce LSCs self-renewal through autocrine and paracrine pathways, as well as contributing to immune evasion ability [13, 14]. Notably, evidence suggests that no single marker is sufficient to fully identify the phenotypic and functional diversity of the LSCs compartment. Combination marker strategies, integrating antigens such as CLL-1 and Tim-3, may improve the discrimination between LSCs and normal HSCs and increase the sensitivity of residual disease detection beyond standard MRD assays [10]. A deeper understanding of LSCs persistence, immune escape, and therapy resistance, together with standardized and high-sensitivity methods for their detection, is therefore essential to refine relapse risk stratification in AML patients and support the development of therapeutic strategies specifically targeting the compartment that causes leukemia.

OBJECTIVES

This project aims to identify and to characterize LSCs at diagnosis in AML patients referring to the Siena Hematology Unit, using specific immunophenotypic markers such as CLL-1 and Tim-3, monitoring patients after induction therapy and at relapse, if any.

Furthermore, our focus is to acquire a platform of data including rate, quantity and phenotype of LSCs useful to define a personalized LSCs–based MRD (LSC-MRD) assessment for all AML patients. Additionally, we will evaluate the concordance of this LSC-MRD approach with standard MRD assessment methods, including conventional flow cytometry and molecular MRD, as well as with clinical response. Finally, we aim to investigate the prognostic value of LSC-MRD in patients who are MRD-negative by flow cytometry and in those for whom molecular MRD assessment is not evaluable.

MATERIAL AND METHODS

EDTA anticoagulated fresh BM samples of AML patients were used for LSCs characterization after written informed consent in accordance with the Declaration of Helsinki. Briefly, after centrifugation, lysis step, 100 μl of BM aspirate were incubated for 15 minutes at room temperature with the following fluorochrome-conjugated anti-human antibodies: CD45, CD34, CD38, CD90, CD45RA, CD123, CLL-1 and TIM-3. For each sample, corresponding isotype controls were used. AML-LSCs identification was performed with the BD FACSLyric flow cytometer and data were analysed by using BD FACSuite software (BD, Biosciences). AML-LSCs were identified by gating on CD34+CD38-CD45dim population and then evaluating the positivity for CD45RA, CD123, CLL-1 and TIM-3 antigens. The negativity for CD90 antigen discriminated LSCs to HSCs (Figure 1). Molecular and cytogenetic analysis have been performed to classify AML patients into favourable, intermediate and adverse prognostic risk, according to ELN guidelines [2].

Furthermore, patients' charts were manually reviewed to collect data, including demographics, disease characteristics at the baseline and after induction therapy and their outcomes. All data were entered into a database, and they were handled in accordance with local regulations.

Definitions

According to 2022 ELN guidelines MFC-MRD negativity is defined as < 0.1% of CD45 expressing cells with the target immunophenotype, among total white blood cells (WBC). [2]

Finally, according to ELN-DAVID AML working party, NPM1 and CBF are considered MRD negative when mutNPM1/ABL1 or mutCBF/ABL1 are <0.001% or undetectable using qPCR, defined as cycling threshold ≥ 40 in ≥ 2 of 3 replicates and almost ABL1 ≥10000 copies. [15]

RESULTS

Patient's characteristics

From November 2022 to October 2025 LSCs presence has been investigated in 58 AML patients, of which 40 were analysed from diagnosis, while 18 patients were studied during consolidation therapy. This thesis will focus on the 40 AML patients studied at diagnosis and during treatment.

Regarding patients' characteristics 24/40 (60%) were male, while 16/40 (40%) were female, with a median age of 68 years [28-83 years]. In our population 20/40 (50%) patients had de novo AML, while 20/40 (50%) had secondary AML (1 therapy related, 16 secondary to myelodysplastic syndrome and 3 secondary to chronic myeloproliferative neoplasm); 23/40 (57,5%) received high dose chemotherapy, while 17/40 (42,5%) received hypometilating agents as induction therapy, according to their fitness and comorbidities. When considering ELN risk classification, 15/40 (37,5%) were high risk AML patients, 19/40 (47,5%) were intermediate risk and 6/40 (15,0%) were low risk.

The baseline cell blood count showed a median white blood cell of 4365/mmc [810-161000], with 39% [0-78] of peripheral neutrophils and 6% [0-100] of peripheral blast cells. The median haemoglobin was 9.0 g/dl [8.0-9.9] and the median platelets count was 40000/mmc [18000-167000]. Finally, LDH value was increased in 24/40 (60%) patients, with a median value of 268 UI/L [254-1786].

Considering baseline cytogenetics, karyotype showed alterations in 23/40 (57,5%) AML patients while it was normal in 17/40 (42,5%). Furthermore, analysing molecular alterations, we found 33/40 (82,5%) patients with WT1 overexpression and 7/40 (17,5%) with a normal value WT1, with an overall median value of 3170 copies [304-33804 copies]. In 7/40 (17,5%) patients we found FLT3 ITD mutation, in 6/40 (15%) NPM1 mutation and 9/40 (22,5%) patients presented other molecular alterations (3 INV16, 2 BCR::ABL1, 2 IDH1, 1 IDH2). Finally, 20/40 (50%) patients showed NGS genetic mutations, the most represented were SRFS2, RUNX1 and IDH1. Characteristics of the whole population are reported in Table 1.

Among the 40 enrolled patients, 11/40 (27,5%) have been studied only at diagnosis while 29/40 (72,5%) have been studied also after induction therapy and five of them even after the relapse.

Table 1: Whole population characteristics.

Patients	40
Male (%)	24 (60)
Female (%)	16 (40)
Median age (years) (min-max)	68 (28-83)
De novo AML (%)	20 (50)
Secondary AML (%)	20 (50)
ELN risk	
High (%)	15 (37,5)
Intermediate (%)	19 (47,5)
Low (%)	6 (15)
High dose chemotherapy (%)	23 (57,5)
Hypometilating agents (%)	17 (42,5)
Median WBC (mmc) (min-max)	4365 (810-161000)
Median Neutrophils (%) (min-max)	39 (0-78)
Median Blast (%) (min-max)	6 (0-100)
Median hemoglobin (g/dl) (min-max)	9 (8-9,9)
Median platelets (mmc) (min-max)	40000 (18000-167000)
Median LDH (UI/L) (min/max)	268 (254-1786)
Karyotype altered	23/40 (57,5)
Molecular alterations	
Median WT1 (copies) (min-max)	3170 (304-33804)
Overexpressed (%)	33/40 (82,5)
Hypoexpressed (%)	7/40 (17,5)
FLT3 ITD mut (%)	7/40 (17,5)
NPM1 mut (%)	6/40 (15)
Other mutations (%)	9/40 (22,5)
NGS mutated (%)	20/40 (50)

AML: Acute Myeloid Leukemia; ELN: European Leukemia Net; WBC: white blood cells; LDH: lactate dehydrogenase; WT1: Wilms' Tumor 1; FLT3: FMS like tyrosine kinase; NPM1: nucleophosmin 1; NGS: Next Generation Sequencing

LSCs presence at diagnosis

LSCs have been identified in the whole 40 AML patients at diagnosis; although AML blast cells were prevalent, we found a small, yet well evident, amount of LSCs in all samples, and we used the phenotype of these quiescent cells to construct a personalized LSCs MRD for each patient. LSCs were different and quite distinguishable from AML blast bulk, and they were commonly identified by the lack of CD38.

Furthermore, in some AML patients we also found residual normal HSCs, with a CD34+CD38- phenotype distinguishable from LSCs for the CD90 positivity and CD45RA negativity and for the absence of aberrant markers. (Figure 1)

Therefore, in our study we confirmed that at diagnosis, beside the bulk of leukemic clone, LSCs and residual HSCs can be identified and characterized using NGF.

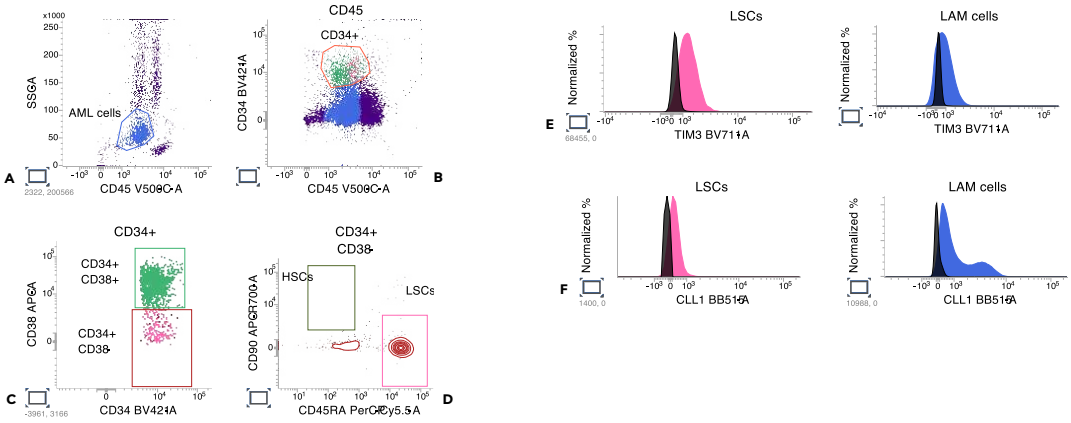

Figure 1: Dot plots demonstrating the gating strategy of LSCs identification in AML sample at diagnosis: a) blasts cells; b) CD34+ cells; c) CD34+CD38- population (pink dots); d) LSCs CD45RA+; e) and f) TIM3 and CLL1 positivity on LSCs (pink histograms) versus blasts cells.

LSCs after induction therapy

Among 29 AML patients analysed post induction chemotherapy, 20/29 (69%) still presented LSCs, instead 9/29 (31%) didn't show LSCs (Figure 2).

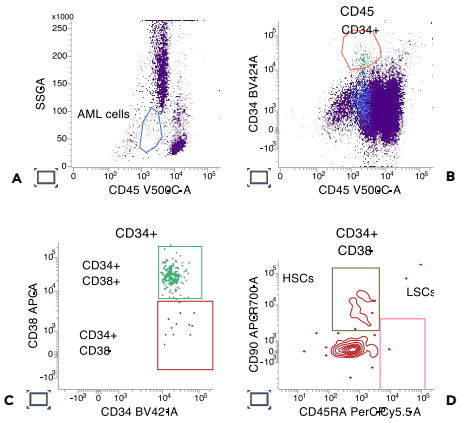

Figure 2: Dot plots demonstrating the gating strategy of LSCs identification in AML sample after induction therapy: a) blasts cells; b) CD34+ cells; c) CD34+CD38- population (red dots); d) LSCs CD45RA+ disappearance.

Furthermore, 12/29 (41,4%) patients achieved a partial response (PR) or a stable disease (SD) after the first course, while 17/29 (58,6%) achieved a complete response (CR). Among them, 5 patients relapsed, presenting positive LSCs at the relapse.

Considering the small and heterogenous sample size of our study, we explored the role of LSCs persistence by comparing this “LSCs MRD” to the residual AML clone (i.e. standard flow MRD) and to the gold standard molecular MRD markers in small specific clusters of patients.

Molecular vs LSCs MRD

In 9/29 (31%) patients evaluated post induction therapy we were able to monitor MRD given the presence at diagnosis of a specific and quantifiable molecular marker (NPM1 mutation, CBFB-MYH11, BCR::ABL1).

Regarding the comparison with LSCs measurement by flow-cytometry we found the following: only 1 patient was negative for both LSCs and molecular MRD, 1 patient was negative for LSCs but molecular MRD positive, 4 patients were LSCs positive with negative molecular MRD and 3 patients were positive for both LSCs and molecular MRD. (Figure 3)

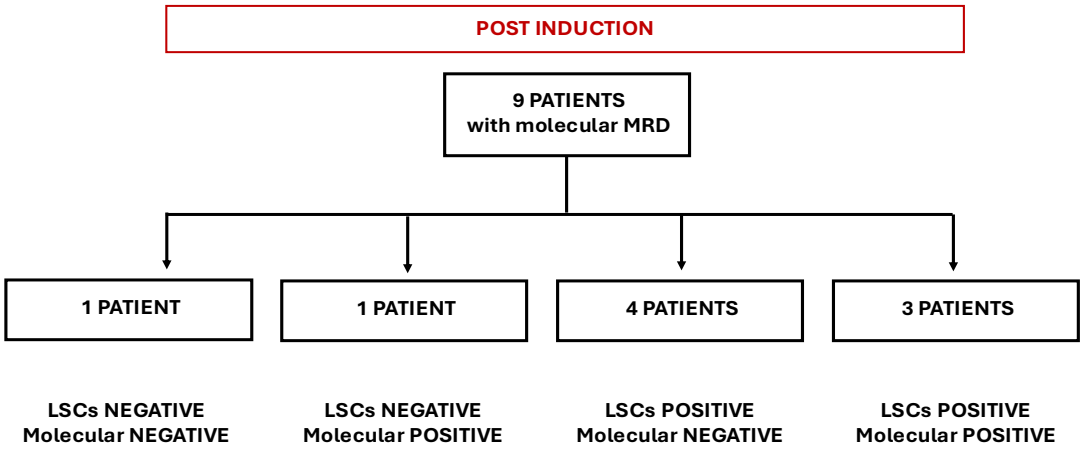

Figure 3: Flow-chart about LSCs presence and molecular MRD.

Flow-MRD vs LSCs MRD

Immunophenotypic MRD was detectable in 12/29 (41%) patients. After induction therapy in two patients LSCs and MFC MRD were concordant: one patient was positive both for LSCs and MFC MRD and one patient was negative both for LSCs and MFC MRD. On the contrary, the remaining ten patients still presented positive LSCs after induction therapy, but negative MFC MRD. (Figure 4)

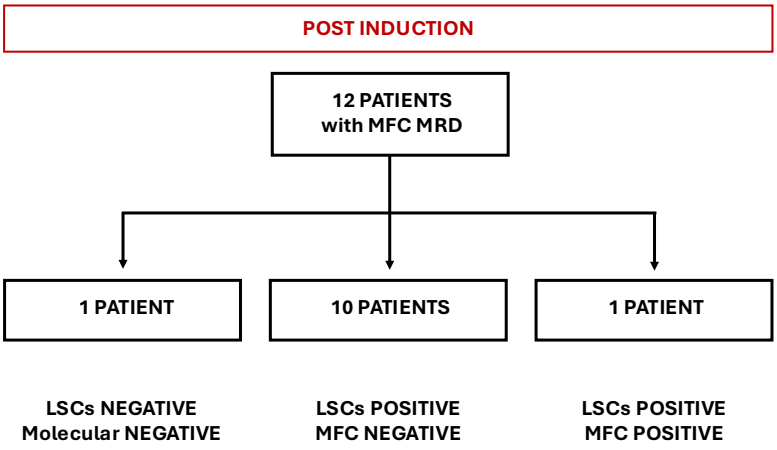

Figure 4: Flow-chart about LSCs presence and immunophenotypic MRD

Molecular MRD vs Flow MRD vs LSCs MRD

Considering molecular and immunophenotypic detectable MRD, only 7 patients were evaluable using both two methods. One patient presented only

molecular positive MRD with negative LSCs and negative MFC MRD after the induction therapy. On the contrary, 3 patients presented LSCs and positive molecular MRD with negative MFC MRD. Finally, 3 patients had only positive LSCs without molecular and MFC MRD. (Figure 5)

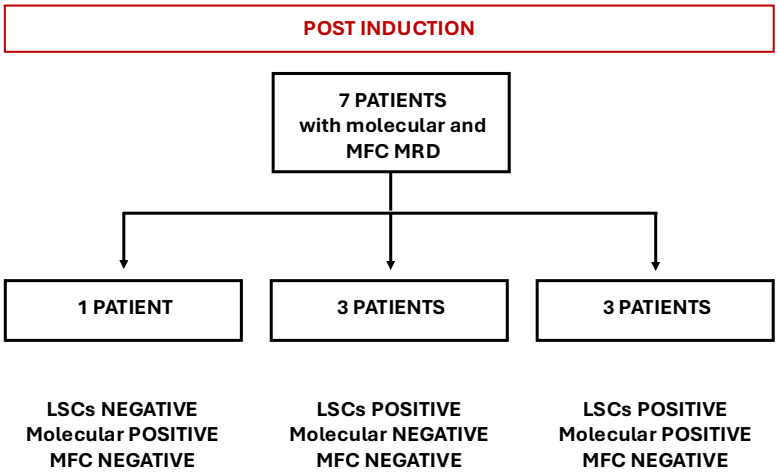

Figure 5: Flow-chart about LSCs presence and molecular and immunophenotypic MRD.

LSCs MRD only

In this series of AML patients, at diagnosis 15/29 (51,7%) showed no measurable MFC nor molecular MRD due to the lack of aberrant surface antigens in the leukemic clone and the absence of molecular markers to monitor with quantitative PCR. After induction therapy we found that 8/15 (53,3%) still had measurable LSCs while 7/15 (46,7%) were LSCs negative, this counting for the whole population, 27,6% (8/29) and 24,1% (7/29) respectively. (Figure 6)

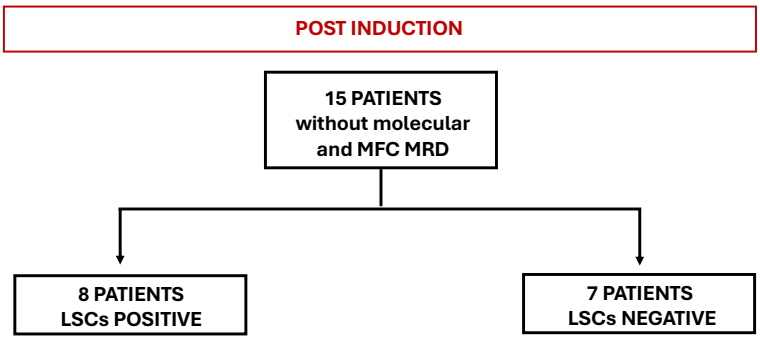

Figure 6: Flow-chart about LSCs presence without molecular and immunophenotypic MRD.

Relapsed patients

Finally, after induction therapy 12/29 (41,4%) patients achieved PR or a SD, while 17/29 (58,6%) patients achieved a CR. Among the latter, 5 patients relapsed (Figure 7).

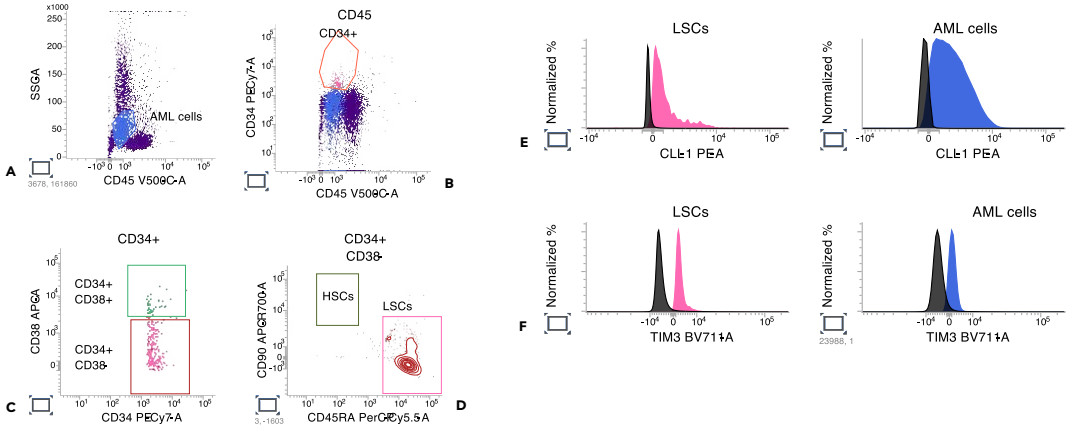

Figure 7: Dot plots demonstrating the gating strategy of LSCs identification in AML sample at relapse: a) blasts cells; b) CD34+ cells; c) CD34+CD38- population (pink dots); d) LSCs CD45RA+; e) and f) TIM3 and CLL1 positivity on LSCs (pink) vs blasts cells (blue).

All the patients at the relapse presented positive LSCs: 3 of them were positive for LSCs also after induction, while 2 were negative. The timeline about LSCs status and MRD status, if any, is reported in Table 2.

Table 2: Relapsed patients.

	Diagnosis	Post Induction	Relapse	Last FU
Patient #1	LSCs POS MFC MRD n.a. Molecular MRD n.a.	LSCs NEG MFC MRD n.a. Molecular MRD n.a.	LSCs POS MFC MRD n.a. Molecular MRD n.a.	PD
Patient #2	LSCs POS MFC valuable MRD NPM1 921 copies	LSCs POS MFC MRD 0.01% NPM1 12 copies	LSCs POS MFC MRD 8% NPM1 876 copies	PD
Patient #3	LSCs POS MFC valuable MRD Molecular MRD n.a.	LSCs POS MFC MRD 0% Molecular MRD n.a.	LSCs POS MFC MRD 94% Molecular MRD n.a.	PR
Patient #4	LSCs POS MFC MRD n.a. Molecular MRD n.a.	LSCs NEG MFC MRD n.a. Molecular MRD n.a.	LSCs POS MFC MRD n.a. Molecular MRD n.a.	PR
Patient #5	LSCs POS MFC MRD n.a. Molecular MRD n.a.	LSCs POS MFC MRD n.a. Molecular MRD n.a.	LSCs POS MFC MRD n.a. Molecular MRD n.a.	PD

FU: follow up; LSCs: leukemic stem cells; MFC: multiparametric flow cytometry; MRD: minimal residual disease; POS: positive; NEG: negative; n.a.: not acquired; PD: progressive disease; PR: partial response

DISCUSSION

AML is characterized by a highly biological heterogeneity, in which cellular subclones could origin from the main clone, representing a challenge in MRD assessment and relapse prediction. [1] Current guidelines (2022 ELN) have defined < 0.1% MFC MRD as negative, however 30% MRD negative AML patients' relapse, suggesting the persistence of a hidden leukemia clone. [1, 16] As reported in the latter ELN-DAVID MRD Working Party, MRD negativity is not like disease eradication. [15]

In this scenario, LSCs could explain disease persistence and relapse, because they are characterized by self-renewal, quiescence and often resistance to standard therapies and they can switch from a sleeping state to an active state, initiating leukemia. [3,4,17,18] Therefore, in AML treatment, tumor burden eradication could be insufficient and could be important to characterize LSCs to evaluate the real residual, and resistant, leukemia burden and to target it with new therapeutic strategies. [19, 20] Despite that, according to 2022 ELN guidelines, MFC identification of LSCs is still investigational, although it is recommended in some clinical trials. [2] Indeed, LSCs have a higher expression of surface markers compared to HSCs, with minimal differences, but their detection by MFC has been very difficult because patients' heterogeneity and the lack of a MRD assay that fits all. [21,22]

In our study we identified LSCs in all 40 AML patients analyzed at diagnosis, confirming that LSCs can be found regardless of age, ELN risk, type of AML or biological characteristics. Moreover, we characterized LSCs using NGF in the whole population, with the identification of a specific LSCs signature in AML patients, in contrast to traditional MRD that cannot always be assessed.

This is a powerful revolution that could open new perspectives to better understand the impact of AML.

In addition to the accurate characterization of LSCs clone, it's also important to quantify it. Despite our efforts to characterize and measure the presence and the persistence of LSCs in all AML patients studied, we could not yet end up to a standardized number to quantify the LSCs clone due to the biological

AML complexity, but it will be the focus of our future studies. Indeed, as reported by I.M. Mayer at al. in a recent review, LSCs number is directly related to leukemia development. [23]

Considering LSCs after the induction therapy, 69% of patients still presented detectable LSCs, suggesting that chemotherapy is effective in reducing leukemic bulky, without a reduced impact on the LSCs compartment, that could represent an MRD marker even when standard markers are negative.

Furthermore, it's interesting to explore HSCs role after induction therapy: we observed HSCs reappearance in some AML patients, instead in some others we couldn't detect any HSCs despite the reconstitution of normal blood value after therapy. Maybe HSCs delayed reconstitution could reflect LSCs persistence or maybe it could show a persistent alteration of BM microenvironment, still favourable to AML cells, as suggested by previous study. [20,21] Indeed, LSCs in the BM have their niche, altering microenvironment and consequently physiological hematopoiesis. [21] In this scenario, the role of HSCs needs further investigation as a possible favourable prognostic marker.

Analysing our data, a discrepancy between traditional MRD and LSCs MRD comes out: 10 patients were LSCs positive, with negative MFC MRD and several patients had LSCs positive without molecular MRD. These results indicates that LSCs could represent a different population from the leukemic bulk usually monitored by conventional MRD, suggesting that traditional MRD measures leukemic bulk, while LSCs MRD probably measures a leukemic reservoir.

Considering relapsed patients 5/29 overall and 5/17 of those who achieved CR after treatment, they were LSCs positive at the relapse and 3 of them were also positive after induction, suggesting a relationship between LSCs persistence and both resistance and relapse, although in the 2 patients with no detectable LSCs after induction we do not know if LSCs were really disappeared or more probably, or the number of residual LSCs was below the NGF detection threshold

An important benefit of LSCs detection appears to be their universality: even if our study includes only 40 AML patients studied at diagnosis it is noteworthy that LSCs could be identified and characterized in all of them, regardless of the presence of an immunophenotypic or molecular MRD, thus representing a potential powerful NGF MRD marker, especially when there is no other standard method to measure MRD.

On the other hand, in our research there are some important limitations. First, we collected data about a small cohort of patients with shorter follow-up. Moreover, we decided to measure LSCs only after the first course of chemotherapy, which could be not the best timepoint to understand and analyze LSCs trend. This choice could partly explain the discordance between LSCs MRD+ and standard flow or molecular MRD+ that we found after induction. For this reason, in the future we will evaluate LSCs in BM collected during all different phases of the treatment and even after BM transplantation, if any, to better understand their trend and to improve their use in AML prognostication and in the prediction of AML relapse. However, the post induction timepoint is important because we observed HSCs reappearance, that could be a potential prognostic predictive factor because it could reflect a favourable BM microenvironment. Finally, we have to better quantify LSCs MRD, trying to find a significant threshold.

CONCLUSIONS

In conclusion, this is an exploratory pilot study confirming LSCs as biological possible additional MRD marker to monitor AML patients during and after therapy.

Furthermore, using a combination between standard immunophenotypic, molecular MRD and LSCs MRD, we could refine prognosis of AML patients, identifying patients who need more aggressive treatments, and maybe it could help the development of personalized target treatments, with the aim to eradicate the reservoirs of disease and to prevent relapse, improving AML patient's outcome.

In the future we will increase the cohort of patients, to acquire a solid platform of data with the ultimately intent to identify LSCs with specific patient signature able to monitor even more accurately this leukemic reservoir. Meanwhile, we will prolong the follow-up to define LSCs prognostic value in MRD and to establish if their quantification over time, and not only after treatment, could become a useful biomarker.

TABLES AND FIGURES

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