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Acute Leukemias

FROM CELLS TO VESICLES: NEXT-GENERATION MEASURABLE RESIDUAL DISEASE TRACKING IN ACUTE MYELOID LEUKEMIA WITH NGS AND DIGITAL PCR

A. Leoni[1|2], M. Farina[2], E. Borlenghi[3], L. Garuffo[1|2], B. Xhahysa[1|2], A. Cavalleri[1|2], F. Trenta[1|2], F. Re[1|2], S. Mutti[1|2], E. Morello[2], D. Avenoso[2], D. Russo[2], M. Malagola[2], S. Bernardi[1|2]

[1]Centro di Ricerca Emato-Oncologico AIL, ASST Spedali Civili di Brescia; [2]Unit of Blood Diseases and Bone Marrow Transplantation, Cell Therapies and Hematology Research Program, Department of Clinical and Experimental Science, University of Brescia, ASST Spedali Civili di Brescia; [3]Hematology Unit, ASST Spedali Civili di Brescia.

Acute Myeloid Leukemia (AML) is a hematologic malignancy characterized by marked biological, clinical, and prognostic heterogeneity driven by genetic and cytogenetic alterations. Although current therapies induce remission in 60-80% of patients, long-term survival remains limited due to frequent disease relapse. Measurable residual disease (MRD), reflecting the persistence of leukemic cells after treatment, represents a key prognostic determinant. Conventional MRD assessment, based on bone marrow (BM) or peripheral blood (PB) analysis using molecular assays and multiparameter flow cytometry (MFC), is limited by restricted applicability and variable sensitivity. As previously demonstrated, Extracellular Vesicles (EVs), actively released by leukemic cells into the bloodstream, carry nucleic acids that reflect the tumor's mutations landscape. Their stability and molecular cargo make them a promising, minimally invasive and sensitive source for MRD monitoring.

We conducted a feasibility study to evaluate an integrated NGS-driven dPCR approach for detecting and longitudinally monitoring AML-associated mutations across BM, PB and EV derived DNA.

Targeted NGS Myeloid panel (SOPHiA Genetics) was used at diagnosis to identify AML-related mutations, which were then monitored by dPCR using custom-designed (78%) or commercial (22%) TaqMan assays. Clonal hematopoiesis related mutations were excluded. Feasibility was predefined as the ability to monitor ≥70% of patients with this workflow.

NGS identified at least one trackable AML-related mutation in 22 of 25 patients (88%), all of whom were successfully monitored by dPCR, thus exceeding the feasibility threshold. dPCR achieved high analytical sensitivity (up to 10^{-5}), and presented excellent concordance between BM, PB, and EV compartments. Compared with RT-qPCR (52%) and MFC (56%), the NGS-driven dPCR strategy significantly expanded MRD coverage and sensitivity, enabling monitoring even in patients lacking standard markers. dPCR-MRD positivity, observed across BM, PB, and DNA-EVs–derived samples, anticipated relapse in 9 of 10 cases (median lead time, 4 months) (e.g., Figure 1) and correlated with inferior survival outcomes.

EV-MRD status showed strong concordance with BM and PB, and presents a clear signal with reduced background noise, supporting the potential integration of EV-based monitoring into routine clinical practice. Overall, this study demonstrates that the EV-based NGS-driven dPCR approach is feasible, highly sensitive, and broadly applicable strategy for next-generation MRD monitoring in AML. In fact, EVs actively released from leukemic cells enables better reflection of residual disease biology, even at low tumor burden, allowing reliable and early detection of molecular relapse. By bridging the breadth of NGS with the precision of dPCR, this approach advances the concept of liquid biopsy in AML and highlights EVs as a powerful and clinically actionable source for dynamic MRD evaluation.

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Fig. 1