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Chronic myeloid leukemia

TRACKING RESIDUAL CML: COMPLEMENTARY INSIGHTS FROM CD26⁺ STEM CELLS AND EXTRACELLULAR BCR::ABL1

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder driven by the *BCR::ABL1* fusion gene. Although tyrosine kinase inhibitors (TKI) have revolutionized disease management, leukemic stem cells (LSCs) persist, sustaining minimal residual disease and relapse. A subset expressing the CD26 membrane marker represents a population of proliferating LSCs detectable in bone marrow and peripheral blood. Parallelly, extracellular vesicles (EV) have emerged as promising circulating biomarkers, as they carry *BCR::ABL1* transcripts protected within their lipidic membrane. However, the relationship between residual CD26+ LSCs and EV-associated *BCR::ABL1* remains unclear. This study aimed to explore the correlation between circulating CD26+ LSCs and vesicular BCR::ABL1 transcripts as complementary indicators of residual disease activity in CML.

Peripheral blood (PB) from 44 adult CML patients in at least major molecular response under TKI therapy was analyzed. Circulating CD26+ LSCs were quantified by multiparametric flow cytometry on the CD45+/CD34+/CD38- population using four-color staining. The extracellular vesicle-enriched secretome (EVES) was isolated from plasma and characterized by Western blot, colloidal nanoplasmonic assay, and atomic force microscopy. Vesicular *BCR::ABL1* transcripts were quantified by digital PCR (dPCR) and compared with *BCR::ABL1* levels in PB cells.

EVES characterization confirmed vesicular particles expressing CD63 and FLOT-1 (Figure 1A), with minimal soluble protein contamination (Figure 1B) and typical spherical morphology (Figure 1C). The median number of circulating CD26+ LSCs was 0.00625 cells/µL (range 0-0.1565), with 32% of patients showing undetectable levels. Median EVES *BCR::ABL1* was 0.230 copies/µL (range 0-0.790), with 14% undetectable (Figure 1D). No correlation was found between CD26⁺ cells or EVES BCR::ABL1 and molecular response, BCR::ABL1 IS%, or dPCR values in PB cells, nor with age or therapy duration (Figure 1E). A significant inverse correlation was observed between CD26+ LSC counts and vesicular *BCR::ABL1* transcripts ($r = -0.39$, $p = 0.0085$), even stronger in deep molecular responders ($r = -0.45$, $p = 0.0079$) (Figure 1F). Patients in treatment-free remission showed higher CD26+ LSC counts, whereas EVES *BCR::ABL1* tended to be higher in those under TKI treatment (Figure 1G).

Circulating CD26+ LSCs and vesicle-associated *BCR::ABL1* transcripts show an inverse relationship, reflecting complementary aspects of residual leukemic activity in CML. As CD26+ LSCs decline, vesicular *BCR::ABL1* may increase, possibly indicating activation of alternative leukemic compartments or enhanced vesicular secretion. Combined monitoring of these cellular and vesicular biomarkers may improve detection of residual disease and provide new insights into CML biology. Larger studies are needed to validate these findings and define the biological and prognostic significance of *BCR::ABL1*-loaded vesicles.

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