

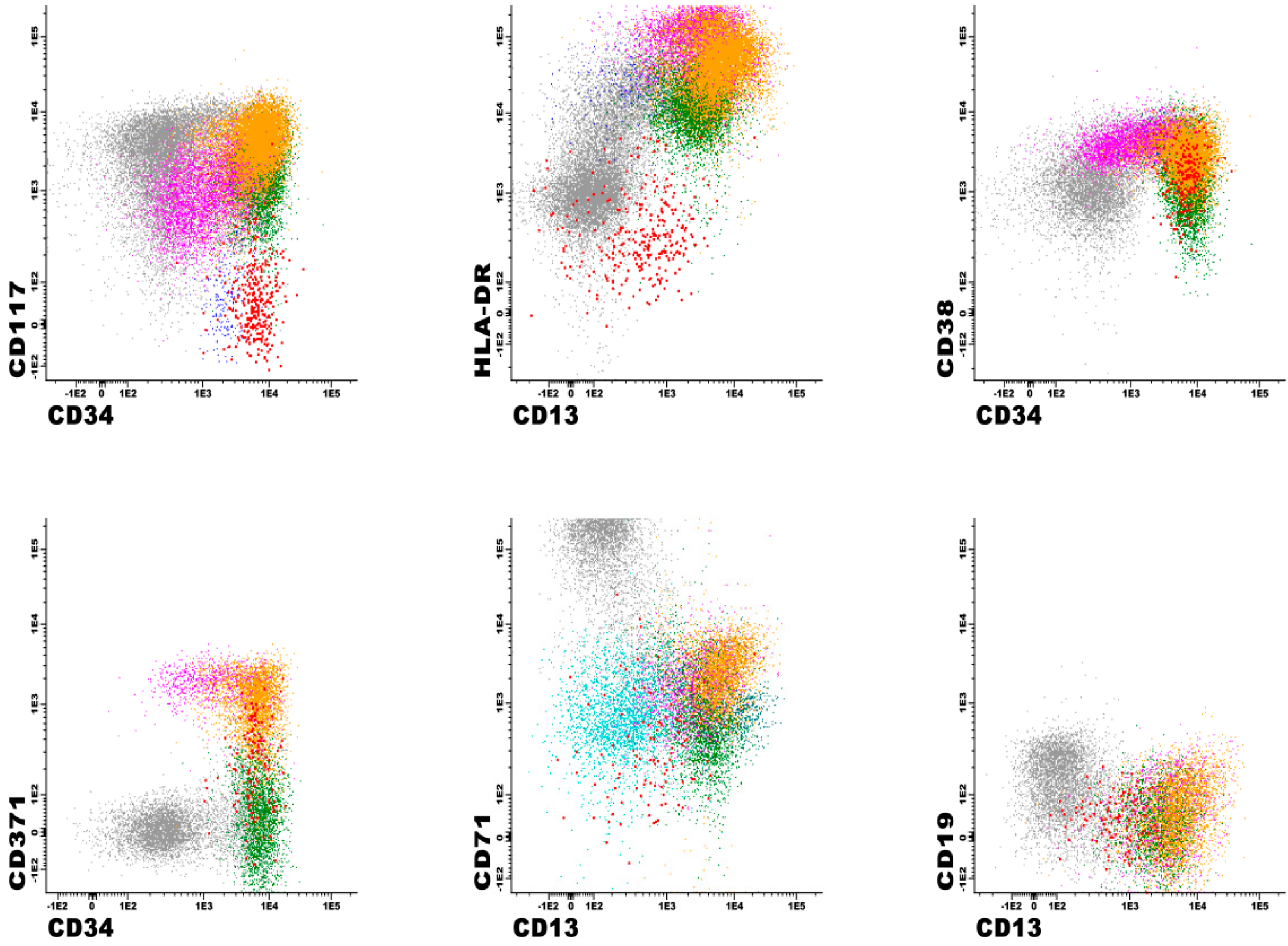
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Lineage assignment, blast diversity & the transition to MRD

CFCM / CLINICAL POSTER SERIES / SPECIALIST CLINICAL FLOW CYTOMETRY

Diagnostic orientation and an AML follow-up example. Published plots retain the authors’ gates and colours; no CFCM patient result is inferred.

1 Clinical question & context	2 Lineage evidence	3 13-marker orientation brief
Define the abnormal compartment Assess blood or marrow with morphology, counts, specimen quality and treatment history. A blast gate is a starting hypothesis, not the diagnosis. Do not force a single lineage Consider AML, B-ALL, T-ALL and ambiguous lineage. Resolve separate populations independently and integrate genetics and classification [1,2]. Time-critical discordance A suspected acute promyelocytic leukaemia requires urgent integrated assessment and molecular confirmation; flow alone must not close the diagnosis [2].	Myeloid MPO or convincing monocytic differentiation is lineage evidence. CD13, CD33 or CD117 alone is insufficient for mixed-phenotype assignment [1]. B lineage Use CD19 intensity relative to B-cell progenitors with appropriate additional B markers. Weak CD19 requires stronger corroboration [1]. T lineage Strong cytoplasmic or surface CD3 is decisive in the appropriate setting; CD7 alone does not establish T lineage. Apply WHO / ICC criteria explicitly [1].	Identity / immaturity CD45 CD34 CD117 HLA-DR TdT Myeloid support MPO CD13 CD33 Lymphoid commitment CD19 CD10 cyCD79a cyCD22 cyCD3 Follow-up tubes, not forced compression Add monocytic, megakaryocytic or erythroid investigation as indicated. Surface / intracellular preparation, clones and fluorochromes require local verification. This is a design brief, not a validated single tube.

4 Published dot plots & visual interpretation	5 Gate logic & corroboration
Published AML follow-up: a restrictive gate can miss disease  Figure 3a reproduced from Jum’ah et al. [3], © 2025 authors, CC BY 4.0. Original plots and colours unchanged; layout resized. Post-chemotherapy example, not a diagnostic lineage panel. The red abnormal compartment is described by the authors as CD117 / HLA-DR-negative with reduced CD13, CD38 and CD371. Read the entire pattern rather than accepting one gate [3].	Acquisition before phenotype Review event stability, singlets, debris and viability where measured. Keep abnormal scatter visible until it has been explained. Broad discovery, then refinement Start with CD45 / scatter and multiple lineage / maturation views. Do not require CD34 or CD117 positivity to admit every abnormal event. Back-gate every hypothesis Check suspected cells across scatter, time, singlets and independent marker combinations. Determine whether they form a coherent population. Compare the right baseline At follow-up use both diagnostic LAIP and different-from-normal reasoning. Regeneration and treatment-associated antigen shifts can mimic or obscure disease [4].

6 Diagnostic pitfalls	7 Interpretation	8 MRD handover	9 Concise evidence
Aberrancy is not lineage Cross-lineage antigens are common. Low-intensity MPO or an isolated marker demands context and appropriate controls [1]. Normal precursors Hematogones and regenerating myeloid precursors have structured maturation. A low-dimensional island alone is insufficient. Technical artifacts Dead cells, tandem instability, compensation and spreading can distort dim intracellular or rare-event signals.	State the evidence Describe the abnormal population and supporting lineage markers, meaningful negatives and unresolved alternatives. Integrate, then classify Combine phenotype, morphology and genetics. Do not assign a contemporary genetic entity from a flow surrogate. Document limitations Identify omitted markers, compromised specimens and insufficient event counts; recommend the next diagnostic investigation.	Preserve the baseline Record diagnostic heterogeneity and useful LAIPs, not only the dominant phenotype [4]. Quantification requires validation Use the assay’s validated detection and quantification rules with the actual informative denominator. Low-event findings At or below the validated quantification boundary: detected but not quantifiable when identity and detection are supported. Never promote uncertainty into a precise percentage.	[1] Khoury JD et al. Leukemia 2022;36:1703–1719. doi:10.1038/s41375-022-01613-1. [2] Döhner H et al. Blood 2022;140:1345–1377. doi:10.1182/blood.2022016867. [3] Jum’ah HA et al. Cancers 2025;17:1155. doi:10.3390/cancers17071155. [4] Heuser M et al. Blood 2021;138:2753–2767. doi:10.1182/blood.2021013626. Technical support: Shapiro HM. Practical Flow Cytometry. 4th ed. Wiley-Liss;2003. doi:10.1002/0471722731. No book figures reproduced.

Do not let a narrow blast gate substitute for lineage assignment or a multidimensional search for disease.