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Acute Leukemias

DOUBLE MARKER STRATEGY TO MEASURABLE RESIDUAL DISEASE ASSESSMENT IN PHILADELPHIA-LIKE ACUTE LYMPHOBLASTIC LEUKEMIA

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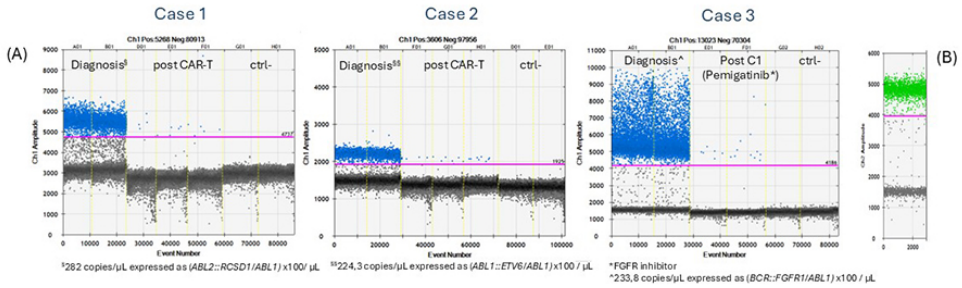
Introduction: Philadelphia-like acute lymphoblastic leukemia (Ph-like ALL) exhibits a gene expression profile resembling that of Ph+ ALL, furthermore, most cases harbor distinct fusion genes involving tyrosine kinases or cytokine receptor rearrangements. As known, in ALL measurable residual disease (MRD) provides essential prognostic and therapeutic guidance. Currently, MRD is mainly monitored by real-time quantitative PCR (RQ-PCR) targeting clonal immunoglobulin and T-cell receptor (IG/TR) gene rearrangements or flow cytometry–based assays. However, in some cases these methods may have limited sensitivity or applicability. Indeed, in the previous GIMEMA ALL2317 trial, Ph-like ALL cases achieved MRD negativity after blinatumomab administration, but later relapsed, highlighting the need for different markers for MRD monitoring (Bassan R, et al. *Blood* 2025). Given the relatively frequent presence of gene rearrangements in Ph-like ALL, we sought to assess MRD using digital droplet PCR (ddPCR) based on patient-specific fusion transcripts and to compare it with MRD analysis by IG/TR RQ-PCR to explore their prognostic and predictive significance.

Methods: The Ph-like gene expression signature was determined using the BCR/ABL1-like predictor tool (Chiaretti S, et al. *Br J Haematol* 2018). At diagnosis, targeted RNA sequencing (RNA-seq) was performed to identify patient-specific genetic lesions. Whenever feasible, primers and probes were designed based on the fusion transcript sequences identified by RNA-seq to enable MRD assessment by ddPCR. For each fusion gene, a FAM-labeled probe was designed, while the *ABL1* gene, labeled with HEX, served as a reference to calculate the target-to-control gene ratio. DdPCR was carried out as previously described (Ansuinelli M, et al. *Hematol Oncol* 2021). MRD was also monitored by IG/TR RQ-PCR.

Results: RNA-seq identified patient-specific fusion genes in 51 of 95 Ph-like ALL cases (54%), including 18 cases with ABL-class rearrangements. So far, ddPCR-based MRD analysis has been performed in 3 cases of patients relapsed after transplant, harboring different fusion transcripts (*ABL2::RCSD1*, *ABL1::ETV6*, *BCR::FGFR1*). DdPCR at previous time-points resulted in 3 positive signals. At the same follow-up, IG/TR RQ-PCR was positive - with a 1-log difference - in one case, positive not quantifiable (PNQ) and negative in the remaining cases (Figure 1).

Conclusions: Our findings support the use of ddPCR based on patient-specific fusion genes as a robust, sensitive, and disease-specific tool for MRD monitoring, potentially improving risk stratification and therapeutic decision-making in Ph-like ALL patients. By designing patient-specific assays targeting leukemia-associated fusion transcripts, ddPCR enables precise MRD measurement even at very low disease levels. Additional patients are undergoing this dual monitoring strategy also in other settings (such as *SET::CAN* subgroup in T-ALL).

Figure 1. A, Plot showing ddPCR result of the MRD analysis evaluated in Ph-like ALL patients with *ABL2::RCSD1*, *ABL1::ETV6* and *BCR::FGFR1* fusion transcript respectively (droplets in FAM). **B,** *ABL1* control gene amplification (droplets in HEX). **C,** Table showing MRD results with two different markers (IG/TR vs fusion transcript).



(C)

	IG/TR (VH3-JH4) by RQ-PCR	*BCR::FGFR1 (ABL2::RCSD1/ABL1)* x 100/µL by ddPCR
Case 1: post CAR-T	5 x 10-4	0,022 copies/µL
	IG/TR (VH1-JH6) by RQ-PCR	***BCR::FGFR1 (ABL1::ETV6/ABL1)* x 100 by ddPCR**
Case 2: post CAR-T	PNQ	0,018 copies/µL
	IG/TR (VH3-JH6) by RQ-PCR	***BCR::FGFR1 (BCR::FGFR1/ABL1)* x 100 by ddPCR**
Case 3: post C1 (Pemigatinib)	Negative	0,017 copies/µL